

SOIL SCIENCE

RUTGERS COLLEGE

VOL. III

NEW BRUNSWICK, N. J., MARCH, 1917

NO. 3

A REVIEW OF INVESTIGATIONS IN SOIL PROTOZOA AND SOIL STERILIZATION¹

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INTRODUCTION

Soil fertility, which may be broadly interpreted as "the crop-producing power of any soil under given climatic conditions, representing the resultant of many forces, often opposed to each other," has been the subject of considerable speculation and investigation for a period of time extending from the days of the Romans—Cato, Varro, and Virgil, down to the present. With its belated development, supported on the one hand by soil physics, and on the other by agricultural chemistry, which is assuming ever-increasing importance, the agriculturist is familiar. But there remains the developing science of soil biology, until recently unknown, which must do its share in solving the baffling problems of soil fertility. It is true that soil bacteriology, in its 35 years of existence, has advanced with rapid strides, and that it represents practically all of our knowledge of soil organisms, yet the time is now ripe for a more thorough investigation of those other minute inhabitants of the soil—the protozoa, fungi, and algae.

It remained for Russell, Hutchinson, and their associates at the Rothamsted Experiment Station, to furnish the impetus necessary to stimulate experimentation concerning the relation of soil protozoa to bacteria, and its bearing upon soil fertility. This was accomplished largely through the medium of partial sterilization.

As is only natural under the circumstances, there has been no adequate historical review of the literature dealing with soil sterilization and soil protozoology, with the exception of certain introductory briefs published in connection with investigations along one definite line or another.

¹This article, in its original form, appeared in July, 1916, in the *Centralblatt für Bakteriologie* [etc.]. Copies of this journal are not available in the United States. The editors have, therefore, made an exception in this instance to the rule that no papers which have appeared elsewhere are acceptable for publication in *SOIL SCIENCE*. The demand for the valuable data contained in the paper, and the fact that it has been brought up to date by the authors, seem to justify such action.

²Appreciation is expressed to Dr. H. Clay Lint, joint author of the original paper bearing this title, for his valuable work in this connection.

Consequently, it appeared advisable to present something of a survey of the subject to date, not only as an introduction to the investigations to follow; but likewise as of inherent value to the investigator pursuing specialized work along these and allied lines, and to those whose interests may lead them into the field of soil fertility.

The practice of sterilizing soils by heat, as well as antiseptics, has been the basis of no small amount of experimentation before the publication of Russell and Hutchinson's work. However, in view of the importance of the latter, it seems desirable arbitrarily to divide the investigations in sterilization into two parts, treating first of those prior to Russell and Hutchinson, and second of those following. Again for the purpose of a more coherent résumé, soil sterilization may be considered under the two topics of sterilization by heat, and antiseptics, each of which may be further subdivided into three parts, namely: (a) the effect on the physical and chemical condition of the soil; (b) the effect on plant growth; (c) the effect on the biological activities.

SOIL STERILIZATION

The Effects on the Soil of Sterilization by Heat

Until the work of Frank (86) in 1888, soil investigators did not consider that the process of sterilization of soils by heat effected any changes either in the mechanical nature or chemical composition of the soils so treated. In attempting to explain the increased yield observed on sterilized soils, he decided that the steam heating of the soil had caused some chemical changes; and found that heated soils contained a great deal more soluble matter than the unheated soils; the moor soils contained more than twice as much, and the sandy soils not quite twice as much. The increased productiveness was, therefore, to be explained by the fact that by the process of sterilization more material was rendered soluble and made available for plant-food. In experiments of the same general nature, Schmoeger (276) found that from a soil which had been heated in an autoclave at 140° to 160° C. for several hours, he could extract with 12 per cent hydrochloric acid almost as much phosphate as was obtained from the ash of the soil; and much more than could be obtained in the same way from the soil unheated. He concluded that a part of the phosphoric acid existed in the soil in the form of lecithins or nucleic acids. Liebscher (197) also, concluded that the sterilization of cultivated soils by steaming makes the phosphoric acid content more soluble, as well as causing one portion of the nitrogen to escape, and further making another portion of the nitrogen more available for plant growth. He regards the process of sterilization in the light of a nitrogenous fertilizer.

These results were corroborated by Krüger and Schneidewind (190). Hasenbäumer, Coppenrath and König (126), and Pfeiffer and Franke (245), who steamed soil at one atmosphere pressure for 3 hours. The

soil so treated produced a crop of greater dry weight and higher nitrogen content than the unheated, even when the sterile soil was inoculated by portions of fresh, showing that there is an increase in assimilable nitrogen.

Richter (253) employed an air-dried garden soil of medium humus content, and heated 500 gm. at 100° C. for 6 hours on 3 successive days. Another portion was treated in the same manner after first being moistened with 100 c.c. of water (the author omitting to state what percentage of saturation this represents). His results were as follows:

	Unsterilized Soil	Sterilized Air-dried Soil	Sterilized Soil after Addition of H ₂ O
Moisture content	8.36%	2.46%	7.45%
Volume weight: 100 c.c. wet soil has air-dry soil	84.6 gm.	98.8 gm.	90.7 gm.
Specific gravity	2.376	2.380	2.359
Porosity: 100 c.c. air-dry soil has actual soil	35.60 c.c.	41.10 c.c.	38.45 c.c.
Capillary rise of water—			
12 hours	20.5 cm.	21.50 cm.	19.5 cm.
24 hours	24.0 cm.	26.27 cm.	24.0 cm.
48 hours	28.0 cm.	30.32 cm.	26.0 cm.
72 hours	30.0 cm.	32.34 cm.	29.5 cm.
Absorption of 100 gm. air-dry soil	67.89 gm.	72.89 gm.	60.80 gm.
100 gm. air-dry soil absorb NH ₃	112.4 mg. N	109.7 mg. N	96.10 mg. N
Total N in 1 kg. air-dry soil	5.260 gm.	5.320 gm.	5.325 gm.
As NH ₃	0.042 gm.	0.068 gm.	0.088 gm.
100 gm. air-dry soil gives H ₂ O extract	0.378 gm.	0.646 gm.	0.690 gm.
Organic extract	0.147 gm.	0.363 gm.	0.409 gm.
Inorganic extract	0.231 gm.	0.283 gm.	0.281 gm.

The sterilized air-dry soil exhibits a variable absorbing capacity for water as regards capillary rise, for many portions of the sample remain dry. Although the total nitrogen content remains the same, a portion of the nitrogenous material is transformed into an available form, especially where water has been added to the soil previous to sterilization. Furthermore, a large amount of organic matter is made available. In some cases there is even a doubling of the amount of water-soluble organic matter.

König, Hasenbäumer and Grossman (180) attempted to correlate the fertility of soils with the effects produced in them by steaming under pressure of 5 atmospheres. From their results they conclude that the more easily and largely the organic matter decomposes under this treatment the more easily and extensively the plant-food of the soil becomes available to plants.

This theory finds further support in the work of Stone and Smith (296, 297, 298), Stone and Monahan (299, 300), Stewart (305), Rudd (256) and Selby (284, 285). Stone (300) and his associates, working with soil partially sterilized, in an effort to destroy insects and fungous diseases, found that a sterilized loam soil with a fair amount of organic matter produced good crops, while a sterilized subsoil produced poor

ones. They have also developed devices for sterilization on a commercial scale for greenhouse work. Gedroitz (97) does not offer any such broad generalization, but makes certain qualifications, by stating that some of the changes in the soil which are caused by sterilization, such as the increased solubility and assimilability of the nutritive substances and the increase of the absorption capacity of the humus, are unqualifiedly useful for the plant; while other changes due to sterilization, may, on the contrary, prove to be unfavorable for the growth of plants. Thus the products of the decomposition of humus, which on some soils are of acid character, may in some cases, be injurious to plants.

Koch and Luken (172) confirm the work of Richter (253) and find that with sandy soils having received applications of fertilizer in each case, the heating produced immediate injurious effects, though these were found to be unimportant if the plants were started in the summer instead of in the spring.

Boyucos (27) indicates how the freezing-point method may be applied to the determination of the concentration of soil solutions before and after steaming. He notes that the effect of the steam heat is to lower the freezing point and consequently the concentration in every soil studied is increased, but that the magnitude of the increase is far greater in those soils in which organic matter is very high or predominates than in the mineral soils. He concludes that apparently steam heat causes a greater amount of material to go into solution in the former than in the latter classes of soil.

Koch (176) has also studied the effect of sterilization of soils by heat and antiseptics as measured by the concentration of the soil solution. The increase in concentration of the soil solution varies with the type of soil and treatment employed, steaming being more effective than the applications of formalin.

A. Wilson (324) has likewise reported experiments in which the depression of freezing points and the electrical conductivities of extracts of soils which had been heated for two hours at from 60°-150° C. It was found that heating increased the amount of soluble matter in soil. There was also a change in soil texture which had a remarkable effect on the retention of water by the soil. These factors are considered to be responsible for the increased productivity of soils thus treated.

Kelley and McGeorge (161), in reporting on the effect of heat on a wide range of Hawaiian soil types, found that the results showed considerable variation. On the average, drying at 100° C. was found to bring about an increase in the water-soluble manganese, lime, magnesia, phosphoric acid, sulfates and bicarbonates. At this temperature an increase in the solubility of potash, silica and alumina was produced in about 50 per cent of the soils examined, but a decrease was observed in the solubility of these elements in some instances. The solubility of iron was decreased in most instances. Heating to 250° C. or ignition produced

effects on the solubility in water similar to those brought about at 100° C. but varying in degree, these being sometimes greater, sometimes less in intensity than those produced at 100° C. Nitrates underwent decomposition with heat, a decrease having been found to take place at 150° C., while at 200° or 250° C. practically total destruction took place. They corroborate the work of previous investigators in noting the effect of soil heating on the production of ammonia, which at 200° C. was formed in abnormally large amounts. Heating to 200° C. caused a loss of approximately 25 per cent of the total nitrogen, largely from the mono-amino acid group.

Peterson (243) found that heating a soil to 100° C. for 5 hours does not increase the solubility of phosphorus in N/5 HNO₃. At 130° C. a small increase takes place and above this temperature the solubility rises rapidly with a rise of temperature, reaching a maximum at about 200° C. However, the solubility of the mineral phosphates does not seem to be increased by heating to 240° C.

Nagoaka (229) found that on igniting soils there was an increase in the solubility of phosphoric acid.

Berthault (17) discusses the beneficial effects of sterilization by antiseptics as obtained by Miége (218).

In an attempt to sterilize soil without radical alteration, Coleman, Lint and Kopeloff (46) found that intermittent sterilization with dry heat at 82° C. caused less change with greater germicidal effect than various chemicals such as toluene, carbon bisulfid, chloroform, alcohol, osmic acid, etc., under pressure and heat.

In concluding the review of the investigations concerned with the physical and chemical condition of the soil as affected by heating (prior to Russell), it may be well to state that in so far as is feasible the classification proposed at the outset will be adhered to, except in cases where homogeneity would seem to suffer at the hands of chronology. Thus it is advisable at this point to mention the work of Czermak (56), who found that heating the soil by steam for 2 hours in succession at a pressure of from 1.5 to 2.5 atmospheres, reduced the soil surface, and consequently the hygroscopicity, by coagulating the soil colloids. It also increased the solubility of nitrogen as a result of purely chemical changes.

Turning now to the effect of heating the soil upon plant growth (and it may be mentioned that the majority of the investigators previously referred to have recorded increased crop yields as a result of such treatment), it is to be noted that Dietrich (63) gives the first account of the bad effects of soil sterilization on plant growth—showing that there is an increase in soluble nutrients, especially nitrogenous compounds in the heated soils, but that there is also a poison formed by the alteration of the organic matter of the soil. According to the amount of this poison and the sensitiveness of the plant to it, the one or the other factor predomi-

nates, and where the plants were less susceptible to the poison an increased absorption of soluble nitrogen by the plants was observed. The addition of calcium carbonate to the soil to be sterilized prevented the poisonous action. These conclusions are reaffirmed by Schulze (280).

An index of the complexity of the subject under consideration is evident from the fact that Pfeiffer, Guttman and Theil (246) find that their results contradict some of their previous work, to wit: sterilization of soil by steam resulted in a decrease of nitrogen in the soil, and did not have a beneficial effect upon plant growth.

G. W. Wilson (326) has recently recorded results dealing with the growth of plants in heated soil.

Johnson (155), in an interesting preliminary communication confirms the results of previous investigators concerning the influence of organic matter on heated soils, but also considers that the absorptive power of the soil is of importance because of its effect on the assimilation of ammonia by plants. The author finds that the percentage of seed germination as well as plant growth is closely correlated with the amount of ammonia in the heated soils studied. The temperature to which the soil is heated is seemingly the most important factor in determining the extent of the injurious or beneficial action. Approximately 250° C. was found to be the most critical temperature in all the soils used.

Owen (235) presents a review of investigations on soil sterilization which is of interest from the practical standpoint.

In considering the effect of heating the soil upon biological activities it is interesting to note that the first investigators to observe the action of heating soils upon the processes of nitrification and ammonification were Deherain and De Moussy (62) in 1896. They prevented nitrification in two soils by heating them at 120° C. in an autoclave for an hour. The sterilized soil, however, when inoculated with a portion of the unsterilized, produced a much larger quantity of nitrate nitrogen than did the unsterilized soil. It was also observed that sterilization had the effect of increasing the ammonia-nitrogen content, while a considerable quantity of carbon dioxide was also produced by the treatment.

It appears strange that prior to the work of Osmun (234) there is no record of the direct effect of sterilization upon the numbers of bacteria in the soil. While his evidence is not pretentious, nevertheless it well merits recognition. He treated one of two boxes filled with soil for half an hour with steam; the other remaining untreated. One week after sterilization samples were taken from each and the bacterial content determined. At the first examination a decided decrease was noted in the bacterial content of the sterilized soil as compared with the unsterilized, but after an interval of two weeks the number of bacteria in the sterilized soil had increased to almost double the number in the unsterilized, and continued to be greater throughout the investigation. Thus the author concludes that steam treatment of soils stimulates bacterial development in them.

The Effects on the Soil of Sterilization by Antiseptics²

While considerable attention has been bestowed upon the physical and chemical condition of the soil as a result of sterilizing by heat, such has not been the case in the investigations involving treatment by antiseptics; where the primary interest appears to be the influence on plant growth or crop yield. In point of fact, Egorow (70) seems to be the only investigator concerned with the physical effect of antiseptics. He finds that (a) the capillary rise of water in soil treated with CS_2 is slower than in the untreated; (b) the moisture content is reduced considerably by CS_2 , especially in peaty soils; and (c) the water-holding capacity of the soil is decreased. Thus he concludes that the treatment of soil with CS_2 acts unfavorably upon the water content of the soil.

Egorow observes from his experiments with etiolated seedlings of *Cucurbita*, *Pepo* and *Helianthus annuus* (using Nabokich's method) that the increased crop yield resulting from the addition of CS_2 (.03 to .06 c.c. per litre of water) is partly due to the stimulating effect exercised upon the plant.

Simultaneously, by Oberlin (231, 232) in Germany, and Girard (99) in France, the theory was set forth that carbon bisulfid increases crop yield. Oberlin showed, moreover, that grape-sick soils could be rejuvenated by the use of carbon bisulfid, and on this he founded his system of grape culture, where fallowing and rotation could be dispensed with in the resetting of vineyards. Behrens (10) showed that the application of CS_2 to an "onion-sick" ground doubled the crop; while Mach (208) found that 200 gm. of CS_2 per square metre augmented the yield of oats, potatoes and beets. The action of antiseptics on the soil as affecting plant growth has been the subject of investigation by Henry (130), who used 400 gm. of CS_2 per square metre and obtained a large increase in the growth of acacias and other plants. Others were Clemens (45), Bernard (16), Johannsen (154), Kegel (159), Jesenko (152), Dumas (66), Krainski (188), Hesselhink van Suchtelen (312), Fruwirth (94), Perroti (242), Miège (218), and Zolla (336).

To overcome soil sickness for lily bulbs, Loew (202) employed potassium permanganate, tricesol, bisulfid of carbon and chloride of lime, the latter proving most effective in increasing flower and seed formation.

In 1898 Wollny (331) stated that no explanation had been found as yet to account for the beneficial influence of carbon bisulfid on the crop-producing powers of cultivated soils; his conclusions, however, were as follows. (a) The application of carbon bisulfid to the soil within the growing season may lead, according to the amount introduced, to a complete destruction of the growing crop, or to a temporary retardation merely, involving a greater or slighter depression in the production of plant substance. (b) The application of carbon bisulfid several months

²A valuable discussion of the problem appears in "A Review of Investigations in Soil Bacteriology" by Voorhees and Lipman (313).

before planting increases, to a very considerable extent, the fertility of the soil. This influence is felt, according to the amount of carbon bisulfid used, through one or several growing seasons, after which (if no manure or fertilizer has been applied meanwhile) a marked decrease in the yields becomes evident. (c) The bacteria concerned in the decomposition of organic substances and the formation of nitrates in the soil, as well as the tubercle bacteria of the legumes, are not destroyed, even by the application of large quantities of carbon bisulfid, but are only hindered in their development.

One of the more important contributions concerning the action of carbon bisulfid finds expression in the "direct stimulation" theory of Koch (168, 169).

It may be asserted that next to Russell and Hutchinson, Hiltner and Störmer (134, 135, 136, 302, 303) occupy the most important position in the study of the effect of treating the soil with antiseptics. And because of the considerable amount of investigation which their conclusions stimulated, it again becomes necessary to telescope the remaining work on the influence of antiseptics upon plant growth and crop yield, with the influence upon biological activities. For the latter question was the primary concern of Hiltner and Störmer, working together at the outset, and later separately. The following conclusions constitute their so-called "indirect" theory of antiseptic action.

(1) By destroying the existing bacterial equilibrium in the soil, the carbon bisulfid opens the way for an entirely new bacterial development. This is achieved through the unequal retardation in the growth of the different groups of bacteria. Hence certain groups become disproportionately prominent, while others are almost entirely suppressed.

(2) The rapid increase in the numbers of the bacteria is followed by a more intense transformation of plant-food substances. Decomposition and fixation processes result in an accumulation of readily available nitrogen compounds utilized by the crops. Hence the action of carbon bisulfid is in the nature of nitrogen action.

(3) The initial suppression of the nitrifying species becomes of advantage in that the nitrogen compounds, simplified by other species, are prevented from being rapidly changed into nitrates and being leached out of the soil.

(4) The more or less permanent suppression of the denitrifying organisms must be regarded as an additional factor favoring plant growth.

The introduction of the poison into the soil at first decimates its bacterial flora, but with the disappearance of the injurious carbon-bisulfid vapors it also encourages a vigorous and long-continued increase of the organisms, resulting in an increase of the store of more readily available nitrogen. It is still to be determined whether this increase is largely due to the fixation of atmospheric nitrogen or to the unlocking of the vast store of combined nitrogen in the soil. It is most probable, however, that

even though one of these processes predominates the other is surely more extensive than it would be in normal soil. The nitrogen thus secured is not at once made accessible to the higher plants, but is at first laid fast in the bacterial bodies. This assumption would best explain the fact that plants growing upon a soil treated with carbon bisulfid show retarded growth, even some time after the application of the latter, and the explanation hitherto accepted that the injury results from the direct action of the poison seems hardly reasonable after our discovery that the most intense bacterial activities are asserting themselves just at that time. The nitrogen fixed in the bacterial bodies is gradually rendered soluble by decomposition processes, and thereby made accessible to nitrification and the higher plants. Hence when the carbon bisulfid is applied in the fall, there is enough time left until the planting of the following spring crop for the mineralization of the bacterial nitrogen. It is quite evident, of course, that the nitrogen combined in the bodies of generations of bacteria is not all made soluble within a single year, but only in the course of several growing seasons, so that we may readily account for the increased harvests secured for two or more successive years after strong applications of carbon bisulfid, even though the bacterial transformations had by that time declined. The exhaustion of the soil finally manifesting itself after a shorter or longer time may be explained by the deep-seated changes in the bacterial soil flora, which does not return so easily to its normal state. It is quite possible that the return to the normal conditions is prevented by the exhaustion for years to come of the more available portions of the plant nutrients.

Heinze (127, 128, 129) and Krüger and Heinze (189) corroborating the work of Hiltner and Störmer (136), as well as that of Moritz and Scherpe (224), who also interpret the increased yield from the antiseptic action of CS_2 as being due to the increased nitrogen supply resulting from the activity of the microorganisms agree that the influence of carbon bisulfid is much like that of a nitrogenous manure.

Heinze states that the action of carbon bisulfid undoubtedly exhibits the same characteristics as that of nitrogenous substances, as is clearly evidenced by the dark green color and the vigorous development of the plants. Subsequently there may be observed a decided tendency of grain crops to lodging, just as if too great quantities of nitrogen were at their disposal. On the whole, we must seek the main course of the beneficial effect of carbon bisulfid on the soil in the enormous increase of soil organisms at the proper time. Various data could be furnished to convince even the most skeptical that there is justification for the assumption that the vast increase in the numbers of the various soil organisms must be followed by a great increase in the store of nitrogen available to plants.

It was further shown by the studies of Krüger and Heinze (189) that the large amounts of nitrogen thus made available to the crops are de-

rived partly from soil sources and partly from atmospheric sources. They not only demonstrated that soils treated with carbon bisulfid showed an increase in their total nitrogen content, but also that this increase was the result of the more vigorous growth of the nitrogen-fixing *Azotobacter* species. Heinze states, therefore, that the significance of carbon bisulfid for the entire nitrogen question may be summarized as follows:

The initial suppression of amid-ammonia formation and of nitrification creates favorable conditions for the development of nitrogen-fixing bacteria, while the subsequent more intense transformation of the bacterial proteids and of other nitrogenous organic substances into amino and ammonium compounds, and the following vigorous nitrification processes, place at the disposal of the plant an abundant and uniform supply of soluble nitrogen compounds. The various organic materials in the soil—such as plant residues, pectins, pentosans, humic substances, etc.—may furnish the carbon food for the *Azotobacter* species, and suitable carbon compounds may also be furnished by algae and molds.

The action of carbon bisulfid as thus examined in detail may help us to understand the peculiar effects at times produced by the turning under of mustard, buckwheat, rye, and of other non-leguminous crops. It has been noted repeatedly that these crops when plowed under in a green state led to a better growth of the following cereal or root crops on nitrogen-poor soils. As Heinze points out, there may have been more or less justification for this belief, so far as the indirect influence of mustard is concerned. It would seem that at times the action of mustard is not unlike that of carbon bisulfid in affecting the bacterial flora of the soil, and it really appears from facts already known that the green mustard substance in the soil retards the development of the acid-forming species and encourages the growth of the nitrogen-fixing *Azotobacter* species. Heinze thinks, therefore, that further study may enable us to make extensive use of mustard as an indirect source of combined nitrogen, and tries to find theoretical support for his belief in the fact that allyl mustard oil, $C_3H_5-N=C=S$, a constituent of the mustard plant, may be regarded as a derivative of carbon bisulfid.

Gostini (108) and König (179) discuss the various reactions taking place in the soil upon the addition of carbon bisulfid.

Wagner (314) and Morgen (223) found that carbon bisulfid kills denitrifying organisms, while Kurzwelly (193) noted that spores of fungi were able to withstand various concentrations of carbon bisulfid.

Fred (90, 91) also finds that the application of carbon bisulfid increases the soluble compounds of nitrogen and sulfur as well as the bacterial activities. However, the data show clearly that CS_2 does not act alike in all soils or toward all crops.

Du Buisson (34), investigating the extraction and saturation of soils with volatile antiseptics, found that there was a beneficial effect upon the

first as well as the second or residual crop. The volatile antiseptics enhanced ammonification and inhibited nitrification, but no effect was noted after two crops had been grown. There was a tendency for the water-soluble salts to increase. No marked differences were observed as to plant growth and biological activity between the saturation and extraction methods of applying volatile antiseptics to the soil. By the extraction of soil with alcohol, a substance was removed which was toxic in water cultures, but not at all toxic when in the soil itself. He concludes that the beneficial influences obtained by treating soil with volatile antiseptics can not be ascribed to a change in physical condition, to a suppression of some toxic material or to a development of acids from the action of the antiseptics. Thus, the closely coordinated stimulation of plant and bacterial activity is regarded as pointing strongly towards a biological interpretation of the facts.

To return to the chronological development of the literature concerning the effect of sterilization by antiseptics on the biological activities of the soil, it is to be noted that Warrington (320) in his original investigations on the biological nature of nitrification, observed that when air containing carbon bisulfid was passed through the soil the process was inhibited.

In 1894 C. de Briailles (30) treated field plots with 40 and 100 c.c. of carbon bisulfid per square meter and thereafter made monthly determinations of the nitrate content. He noted that during the winter the CS_2 seemed to exert a harmful influence on the accumulation of nitrates. However, with the first open weather in spring the reverse seemed to be true—the CS_2 caused a marked increase in nitrates over the untreated.

Perraud (240, 241) in the same year published evidence to show that CS_2 has a very strong antiseptic action upon the lower organisms—influencing nitrification especially. In practice he recommends the use of organic fertilizers along with this treatment as well as the use of nitrogen in the form of nitrate, to obtain the best results.

Pagnoul (236), working in the laboratory, obtained results similar to those recorded by Perraud (240, 241). He treated soil with 1.5 c.c. of CS_2 per kg., then added a large amount of organic nitrogen in the form of blood meal. He also treated soil with 5 c.c. per kg., and added nitrogen in the form of "Oelkuchenmehl." In the first case nitrification was retarded 15 or 20 days, after which it became more vigorous in treated than in untreated soil. In the second case the retarding effect lasted for a month and vigorous activity was reached only after 44 days.

Koch (170) in a study on nitrogen assimilation, used 100 gm. of soil, 2 gm. of cane sugar, and 20 or 100 c.c. of CS_2 per flask. With 100 c.c. of CS_2 there was a larger loss of nitrogen in the treated than in the untreated samples, in 2 months. Thus he concludes that nitrogen assimilation is weakened rather than increased by CS_2 in soil to which sugar has been added.

In some later work the same author (171) finds in contradiction to Hiltner (135) that the addition of CS_2 does not act as a nitrogenous fertilizer, because the addition of sodium nitrate to plots (yielding an increased crop due to treatment with CS_2) did not have such a stimulating effect.

Upon completion of an experiment similar to that of Egorow (70), Koch is unable to account for the unknown circumstances which determine the effect of CS_2 and ether in effecting the growth of the hypocotyl of the plants used.

In experiments dealing with the addition of ether to the soil, Koch finds that the increased yield is pronounced on the first crop, whereas the residual effect is slight—and as with CS_2 the beneficial effect increases with the amount of application.

This latter fact convinces the author that the increase in yield resulting from treatments is not due to the destruction of harmful bacteria, for otherwise amounts in excess of that required to kill those bacteria would not result in increased yield. Koch's theory of the action of CS_2 remains physiological, *i. e.* stimulation to the plant directly.

Some further interesting data are those presented by Maasen and Behn (207), who find marked differences between the flora of soils in the field treated with carbon bisulfid and soils in containers treated similarly. They also found that CS_2 is detrimental to *Azotobacter*, contrary to the results published by Heinze.

Sirker (290) also furnishes evidence in the cultivation of the mulberry which opposes Hiltner's conclusions. He found that the addition of CS_2 to a completely fertilized mulberry plant increased the vegetation 44 per cent, whereas a heavy application of sodium nitrate was of slight value.

On the other hand, we find other investigators such as Muth (227), who confirms the work of Hiltner and Störmer and considers the application of CS_2 to vine-growing.

Stoklasa (293) regards increased crop yield as a result of CS_2 in the same light as Hiltner and Störmer, and holds that the plants are able to get more phosphate-ions as a result of the disintegration of the bacteria killed by that treatment with CS_2 , and Fautechi (76) both discuss the effect of CS_2 on the germination of various seeds.

Nobbe and Richter (230) obtained increase in crop yields with ether (up to 85 per cent) as well as with other antiseptics.

The controversies growing out of Hiltner and Störmer's work (136), as is observed, are numerous; the consensus of opinion, however, seems to support their conclusions, as for example Lipman and Brown (200) who examined an abnormal soil, applying CS_2 in various quantities alone, and in combination with muriate of potash and acid phosphate, and determined the ammonifying, nitrifying, denitrifying, and nitrogen-fixing powers of the soil.

The results confirm the claim of Hiltner and Störmer that in normal soil flora the different groups occur in fairly definite relations which are evidently disturbed by the addition of CS_2 , which, destroying the bacterial equilibrium, prepares the way for an entirely new bacterial development, whereby certain species become far more prominent than previously. The conclusion is that this applies to the nitrifying and nitrogen-fixing bacteria.

In 1908 Coleman (48) published perhaps the most definite laboratory data up to that date. He criticizes the work of Heinze (*loc. cit.*) quite severely, and the fact that the latter's data present several contradictions supports Coleman's contentions.

He treated 17.5 kg. of a composted soil, rich in nitrates, with 250 c.c. of CS_2 , and determined the nitrates present every 2 weeks, finding that nitrification was almost completely inhibited for a period of 7 weeks. After this time, however, after the ammonia had disappeared, nitrification was much more vigorous in the treated than in the untreated soil. A duplication of this experiment, analyzed weekly, produced identical results. The same experiment repeated with a loam poor in nitrates also confirmed the previous work. Coleman was of the opinion that the strong addition of CS_2 was poisonous, but like most poisons (and this follows the familiar Arndt law) when sufficiently diluted (as it would soon become from constant evaporation) the small amount acted as a stimulant. Thus he concludes that the action of CS_2 at the outset is extremely inhibitory to nitrification in the soil, but later rapidly accelerates it, and consequently CS_2 acts as a stimulant upon the nitrifying bacteria.

Scherpe (274), and others (71, 78, 140, 327), have likewise studied the effect of antiseptics on bacterial activities.

The influence of heat upon soil nitrates has recently been the subject of investigation by Hill (131). In comparing nitrification in sterilized and unsterilized soils to which various additions of green manures had been made, it was found that the nitrate content was very much higher in the sterilized samples. In this case the soil was heated 2 hours in a steam sterilizer at a pressure of 5 pounds.

Despite the contradictory results appearing in the foregoing review, there are certain definite lines of evidence which may be summarized as follows:

By heating, the physical, chemical and physiological, as well as bacteriological, properties of the soils are more or less altered. Especially significant is the fact that there is a considerable increase in the soluble matter in the heated soils not only of inorganic nature, as phosphorus and potash, but even more in the organic matter made soluble; which results in larger amounts of soluble nitrogenous matter available for plant growth.

The use of antiseptics produces important changes in the soil flora,

bearing more particularly on the process of nitrification, which at the outset appears to be inhibited, but subsequently increases with great vigor. Thus the use of antiseptics seems to be quite generally regarded in the light of a nitrogenous fertilizer.

With regard to crop production, sterilization by means of heating or antiseptics results in increased yield, which of course represents a resultant of the various forces previously mentioned. That this is more than a theoretical assumption is borne out by the general use of antiseptics in practice, as for example in vine-growing. Just where the principal value of sterilization lies, whether it be in beneficial chemical changes induced, the direct stimulation to the plant itself, the indirect effect on the biological activities such as the destruction of disease-organisms, or increased nitrification, remains as yet undetermined.

Partial Sterilization

It is evident from the preceding review that the problem of the effects of sterilization both by heating and antiseptics, had hardly reached a comprehensive solution, consequently it is not surprising that the work of Russell and his colleagues should have a profound bearing upon the subject.

The first paper to give any indication of a new mode of attack was that of Russell (257), followed by Darbishire and Russell (60, 61,) in 1907.

In a study concerning the oxidation in soils and its relation to productiveness, the authors found that the absorption of oxygen by soil is mainly brought about by the action of microorganisms, and is greatly diminished if the soil has been previously heated to 120° C. When heated to 95° C. it was found that the rate of oxidation on a sand, two loams and a chalky soil, instead of being reduced was considerably increased, as was the case after treatment with and removal of volatile antiseptics, such as toluene, chloroform, CS₂, etc.

The rates of oxidation of heated soils were as follows:

	Mm. of Oxygen Absorbed in—		
	3 Days	6 Days	9 Days
Hop Garden Soil Unheated.....	3.7	5.2	7.0
Hop Garden Soil Heated to 95° C.....	6.0	8.2	12.0
Garden Soil Unheated.....	7.5	10.2	15.5
Garden Soil Heated to 95° C.....	16.9	27.2	33.2

From results with crops of buckwheat, mustard, turnips, wheat, rye, as well as various leguminous and non-leguminous crops grown in soils heated and unheated, and soils treated with volatile antiseptics and untreated, the authors conclude that the yield of non-leguminous crops is distinctly larger on partially sterilized than on unsterilized soils. Legumi-

nous crops, however, show no increase. Analyses show that partial sterilization causes an increase in the amount of nitrogen, phosphoric acid and potash taken up by the crop, and in the nitrogen and phosphoric acid in the dry matter—in other words it increases the “availability” of these plant-foods, and this phenomenon appears to be connected with the modification of the bacterial flora brought about by partial sterilization. When the soil is heated, however, chemical decomposition also takes place.

It is difficult to approach the admirable work of Russell and Hutchinson (265), without an appreciation of its thoroughness and precision. In taking up a consideration of the effect of partial sterilization of soil on the production of plant-food, they note that when the soil is partially sterilized it becomes more productive and capable of yielding larger crops, as has been previously established. The following experiments and conclusions constitute their theory regarding the explanation of this phenomenon.

It should be stated at the outset, however, that the soil employed in their experiments was taken from an arable field and contained moderate, but not large amounts of nitrogen, organic matter, and calcium carbonate. Partial sterilization was effected either by heating to 98° C. or by addition of 4 per cent of toluene, which at the end of 3 days was allowed to evaporate by spreading out the soil in a thin layer as long as might be necessary.

Turning our attention to the changes which take place in partially sterilized soils, it is found that in the untreated soil there is no accumulation of ammonia, whereas the “toluene evaporated” soil, as well as the soil heated to 98° C., show an increased production of ammonia. That this is mainly the work of microorganisms is proven by the following considerations. (a) The curves belong to the type associated with bacterial, rather than purely chemical. (b) Soil which has been heated to 125° C. (at which temperature all organisms are killed) behaves altogether differently: after the first production of ammonia due to heating there is no further change. (c) If the toluene is left in the soil there is only a slow production of ammonia, and never a rapid rate; the curve is more nearly linear. The action of microorganisms is here excluded, but enzymes may still act. (d) The rapid period sets in only when the soil is sufficiently moist. Thus the two significant changes induced by partial sterilization are, first, an increase in the amount of ammonia; and second, cessation of the nitrifying process.

It now becomes necessary to determine the part played by bacteria, and why they can increase so much more rapidly in the partially sterilized soil (which accounts for the increased ammonia production) than in the untreated soils. That the comparative inertness of the bacteria in the untreated soil cannot be caused by any bacterial factor is evidenced by

the following considerations. (a) If a filtered soil extract containing bacteria from an untreated soil is added to a toluened soil, there is an increase in the rate of ammonia production, and also in the number of bacteria. (b) However, if untreated soil is added to toluened soil, there is no increase, but on the contrary, a reduction. (c) As pointed out above, an extract of the toluened soil is more active than an extract of untreated soil. (d) But when the extract of toluened soil is added to the untreated soil there is no increase in ammonia production.

The conclusion drawn is that "the untreated soil contains a factor, not bacterial, limiting the development of bacteria, this factor being put out of action by toluening or heating."

Having determined the presence of a limiting factor in untreated soils an examination of its nature reveals that: (a) it is not a toxin for if it were it would be sure to affect the nitrification bacteria most; (b) barley seedlings grown in aqueous extracts of untreated and toluened soils showed no difference in growth over a period of 4 weeks; (c) the limiting factor is probably biological, for when untreated soil is added to toluened soil the reduction in the rate of ammonia is not at once operative. It is also a large organism, since it is only in the soil and not the filtered extract of the untreated soil that is effective in reducing the rate of ammonia production in toluened soil. An examination of treated and untreated soil was made, and the latter revealed the presence of large organisms, protozoa, etc., which constitute the factor, or one of the factors limiting the bacterial activity, and therefore the fertility of untreated soil. Direct evidence is furnished by inoculating toluened soil or soil extract with cultures of large organisms and studying the effect produced—which is a consequent depression in the rate of ammonia formation.

Thus the so-called "protozoan theory of soil fertility," may be stated as follows:

The microorganic flora of the ordinary arable soil includes a wide variety of organisms performing very different functions, which may be divided roughly into two classes: (a) saprophytes, tending to increase the fertility of the soil, *e. g.*, producing ammonia, fixing nitrogen, etc., and (b) phagocytes and large organisms inimical to bacteria which limit fertility. Between these two classes of organisms there is an equilibrium under natural conditions, but when partial sterilization takes place the phagocytes are killed but the bacterial spores are not; and subsequently the latter develop with great rapidity, since they are freed from the attacks of their enemies, and there is an increase not only in ammonia but likewise in crop production.

Some additional evidence in support of the theory outlined above is to be found in the work of Russell and Golding (263, 264) and Russell and Petherbridge (267, 268, 269).

In the examination of sewage-sick soils, two distinct sets of causes can be traced at work: physical causes that lead to retarded percolation; and a factor detrimental to bacteria, which is an abnormal development of the factor in every respect similar to that shown by Russell and Hutchinson to exist in ordinary soils, but having more pronounced effect in the sewage-sick soil because of the favorable condition of high amounts of moisture and organic matter.

After the harmful factor is killed by partial sterilization the bacteria multiply rapidly—the application of such a practice being possible on a large scale. The same treatment ameliorates the sickness in glass-house soils, which is partly conditioned by this protozoa factor (268).

In studies on the partial sterilization of soil for glass-house work with chrysanthemums, spinach, radishes, tomatoes, cucumbers and various ornamental plants, the conclusions arrived at were: partial sterilization of soil increases the supply of food for the plant, somewhat alters the growth of the plant, and kills insect pests. It may cause a temporary retardation in germination and in early growth, the amount of which varies according to the nature of the soil, the seed, and general conditions. It did not prove advantageous for pot work where abundant supplies of clean virgin soil and manure were available. It is, however, useful for work with borders, cold frames, and for plants that are to run for some time without manure. It leads to better root development, sturdier and healthier plant, earlier flowering, more prolific fruiting and better quality of fruit. It is particularly useful for commercial glass-houses where soil pests are a source of trouble and "soil sickness" sets in. The most effective method is to heat the soil to a temperature above 140° F. but not exceeding 212° F.

In a later paper, embodying the results of further investigations, Russell and Hutchinson (266) not only corroborate their previous work, but offer the following conclusions as well:

The organisms detrimental to bacteria multiply more slowly under soil conditions, and possess a lower power of resistance to heat and antiseptics. In consequence of these organisms the number of bacteria present in the soil at any time is not a simple function of temperature, moisture content and other conditions of the soil, but depends on the difference in the activity of the bacteria and the detrimental organisms. When partial sterilization is effected, however, then bacterial multiplication is a function of the physical conditions. The increase in bacterial numbers as a result of partial sterilization is the result of an improvement in the soil as a medium for bacterial growth, and not on improvement in the bacterial flora (which individually is less potent).

The authors are careful to point out among various other considerations (too detailed to bear recording in this paper), that where the amounts of ammonia and nitrate present are small, a causal relationship

obtains between the increase in numbers of bacteria and rate of ammonia production. When the amounts of ammonia or nitrogen are large, or there is a large decomposition of organic matter as a result of heat, this relationship does not persist, consequently, much of the data set forth by other investigators as undermining the protozoan theory of fertility, are vitiated.

There are some points, nevertheless, which play a significant part in the protozoan theory of fertility, and which are believed not to have been satisfactorily settled by Russell and Hutchinson. It must be borne in mind that in their work on partial sterilization, they consider the effect upon bacteria and protozoa and no mention of fungi has so far appeared. It is a well-known fact that the spores of fungi can withstand high temperatures—Seaver and Clark (281, 282, 283), also Kosaroff (187) and Kurzwelly (193), show that various species of fungi not found in a living state before sterilization flourish vigorously afterwards. Thus, if fungi are present in the soils employed by Russell and Hutchinson, as shown by their plating records is the case, partial sterilization does not exclude all the biological forms of life present, with the exception of bacterial spores, and furthermore, the essential value of this fact is that many species of fungi are capable of producing considerable quantities of ammonia. This contention is borne out by the results of Müntz and Coudon (226), Marchal (210) and McLean and Wilson (216), which deserve more than passing notice.

Müntz and Coudon (226) were among the first to investigate the production of ammonia from organic matter with the use of soil as a culture medium. They used 5 cultures of bacteria and 2 species of fungi: *Mucor racemosus* and *Fusarium meuntzii*.

A comparison of the amount of ammonia formed in veal bouillon with that formed in soil to which organic matter had been added, was made. Ammonia determinations were made after incubating 17 days, and the fungi, as well as the bacteria, had produced ammonia.

Marchal (210), using portions of egg albumen containing 2 per cent of albumenoid nitrogen as a culture medium, confirmed the conclusions of Müntz and Coudon. He emphasized the importance of having the culture media as nearly as possible of the same nitrogen content as the soil so that the results might be comparable. He found that out of the 33 species of molds and yeasts tested by him, those which developed normally showed a production of ammonia in every case. The amounts of ammonia accumulated in the culture were determined only in the case of 5 species. The most active was *Cephalothecium roseum*, which produced .05 gm. of ammonia nitrogen per 100 c.c. of culture medium in 15 days.

McLean and Wilson (216) in ammonification studies with soil fungi used Sassafras loam, which was decidedly acid, in order to isolate the more common species of fungus flora. Twenty-six species are described.

Two soil types were used, one a gravelly loam with 1200 pounds of CaO "Veitch" lime-requirement, the other red shale neutral soil. The "breaker method" was employed in all the ammonification studies. One c.c. of a liquid culture of the fungus was added to each tumbler containing 100 gm. of soil and 155 mg. of nitrogen in the form of organic matter (cottonseed meal or dried blood). The fungi medium used was water, 1000 c.c.; glucose, 20 gm.; peptone, 10 gm.; dipotassium phosphate, 0.25 gm.; magnesium sulfate, 0.25 gm.

The addition of acid phosphate from 0.25 gm. to 2.00 gm. increased the numbers of bacteria as well as ammonia accumulation; while 3, 4 and 5 gm. decreased the numbers of bacteria markedly without decreasing the ammonia accumulation. The growth of fungi increased with increased addition of acid phosphate, indicating that they may be responsible for the ammonia formed and accumulated, in view of their adaptation to media of high concentration and acidity.

Further experiments on ammonification, acid phosphate and lime being used, again indicate that fungi played an important part, whereas the ammonia accumulation of 5 species of common soil bacteria was decidedly depressed by the addition of acid phosphate.

Using pure cultures of soil fungi there was an increased growth of the mycelia with increased ammonia accumulation (where the addition of acid phosphate caused the increase). The *Moniliaceae* were the most and the *Aspergillaceae* the least, active ammonifiers.

When dried blood was used as a source of nitrogen, acid phosphate was found to increase the ammonia accumulation in the soils by 18 out of 26 pure cultures of the fungi studied. On the other hand, where cottonseed meal was added as a source of nitrogen, ammonia accumulation was increased in only 8 cases.

The results of further investigation on the influence of chemical and physical factors on ammonia formation in soils by fungi, make it appear that the ammonification of organic matter in soils by these fungi depends not only upon the chemical and physical composition of the soil, but also upon the quality of the organic nitrogenous matter present as well as the soluble and insoluble phosphates.

Waksman and Cook (319), Kopeloff (181, 182), Coleman (47) and Waksman (318) have advanced considerable data showing that the ammonifying activities of many species of fungi under a variety of conditions are worthy of serious consideration in soil microbiological processes. It is of importance to note in this connection that evidence has been advanced to show that fungi actually lead an active life in the soil, and that the results quoted concerning the activities have a practical significance. Waksman (315) employing Hagem's (121) method isolated several species of fungi from different soils under various treatments.

Conn (49) considers the method employed by Waksman as inconclusive because by using amounts of 10 mg. of soil he obtained no evidence of mycelial development. Since Conn does not state the types of soil employed one must reserve judgment until such data have been advanced. In discussing a new staining method for microorganisms in the soil, he expresses some doubt as to whether fungi mycelia would be revealed. It would seem that this doubt might be dispelled by inoculating soil with a known number of spores of fungi and then testing for their presence.

Brown (32) has stated that Waksman's results have been corroborated in his laboratories.

That fungi have the power of fixing atmospheric nitrogen has been the subject of investigation by Duggar (65), Pennington (238, 239), Puriewitsch (250), Goddard (102) and others (18, 19, 29, 55, 87, 88, 92, 127, 133, 153, 169, 195, 270, 307). Thus it is evident that fungi are deserving of attention in experimentation involving ammonia production and future communications should take cognizance of this fact, which apparently suffers neglect at the hands of Russell and Hutchinson (266), and which constitutes an objection to their claim that increased ammonia production is the result of increased bacterial activity resulting from the removal of protozoa.

In point of fact they state explicitly in the conclusion to their second paper that "an increase in the rate of production of ammonia does not take place without bacterial multiplication" (with but one cited exception). In going over their work we note the following instances taken from the Journal of Agricultural Science, volume 5, 1913, where increased ammonia production was *not* accompanied by bacterial multiplication.

P. 194: Table XII: Case 1, b	Bacterial Numbers Millions per Gram		N as NH ₃ : Parts per Million of Soil		N as NH ₃ Nitrate: Parts per Million of Soil	
	After 16 Days	After 30 Days	After 16 Days	After 30 Days	After 16 Days	After 30 Days
Treated with CS ₂ ...	27	17	29	33	105	109
P. 203: Table XIV (3)	After 300 Days	After 420 Days	After 300 Days	After 420 Days	After 300 Days	After 420 Days
Toluened and 5% Untreated Soil	75	58	36	40	54	58
P. 206, Table XV (2)	After 21 Days	After 68 Days	After 142 Days	After 21 Days	After 68 Days	After 142 Days
Temperature to which Soil was heated, 100° C.	22	10	10	14	19	33
					23	25
						40

The above figures warrant an explanation by the authors and might possibly be interpreted as being due to several causes, one of which might be the presence of fungi.

Another explanation of the same phenomena might be offered, which is concerned with the foundation of Russell and Hutchinson's work, namely, the differences in the physiological efficiencies of the ammonifying organisms. While we may accept their contention that individually the bacteria are less potent after sterilization than they are before, and that the increased numbers are solely responsible for increased ammonia production, nevertheless, it is a well-known fact established by Lipman (198), Müntz and Coudon (226), Marchal (210), and others (7, 19, 44, 73, 95, 204, 275, 286), that various groups of bacteria vary in their power to produce ammonia. And though it may be admitted for the sake of argument, that the species of *Bacillus mycoides* and *Bacillus subtilis*, for example, are less potent after sterilization; if there are fewer groups of *B. mycoides* organisms (which are supposed to be better ammonifiers than *B. subtilis*) after sterilization, we should expect less ammonia to be produced, than if the *B. mycoides* were predominant and *B. subtilis* suffered most by the treatment. In other words the physiological efficiencies of the many groups of bacteria are known to vary in their power to produce ammonia, and just how each of these groups is affected by partial sterilization, and its consequent bearing on ammonia production is a point which furnishes some doubt as to the validity of Russell and Hutchinson's conclusions.

Another point requiring further attention is the cysts of protozoa. In their second paper (266) they say that "the detrimental factor has a fairly sharp extinction point between 55° and 60° C. and their routine examination of soil for protozoa is described thus: "Soil is inoculated into a 1-per cent hay infusion and left in an incubator at 25° for 4 or 5 days, examination being made periodically for protozoa."

Cunningham and Löhnis (54), show that, depending upon the nature of the medium employed, the first appearance of active ciliates is from 4 to 6 days, and 14 to 20 days for amœbæ, a fact which our own experiments corroborate. Thus when Russell and Hutchinson maintain on the basis of a 5-day examination that no protozoa are present, it may be assumed that some amœbæ are still present in an encysted form. Cunningham found that the cysts of amœba were killed by a temperature of 72° C. in solution. Whether 55° to 60° C. in soil (as used by Russell and Hutchinson) is equivalent to 72° C. in solution is a fact that demands proof.

Recent Work in Sterilization

Bearing in mind the principles underlying the experiments and conclusions of the work of Russell (262, 265, 266) and his colleagues, one is in a better position to understand the work stimulated directly and indirectly by their investigations.

In fact Hutchinson's investigations on "the partial sterilization of the soil by means of caustic lime" (143, 144) are a continuation of the previous work. In the first of these two papers he applies CaO in quantities of 0.1, 0.5 and 1 per cent and notes that in quantities greater than 0.1 per cent there is a distinct initial repressive action on the bacteria. One per cent CaO fails to increase either the bacteria or nitrogen in an arable soil. Five per cent increases both, and 1 per cent inhibits multiplication. With a rich garden soil there was an increase with 1 per cent CaO. The author concludes that in addition to the physical improvement of the soil, as well as the chemical benefits, namely, a neutralization of acidity and liberation of plant-food, calcium oxide has an antiseptic value, intermediate in effect between the volatile antiseptics and high temperatures. A depression of bacterial activity persists until all the oxide has been converted to carbonate, then follows a period of active bacterial growth and increased production of plant-food. Pot experiments confirm the laboratory data. Hutchinson concludes that the inhibitory action of CaO on soil bacteria varies with the soil and is probably governed by the organisms present.

In the subsequent paper Hutchinson and McLennan (145), investigating the relative effect of lime as oxide and carbonate, using 5 different soils, found that the addition of CaO in increasing quantities of 0.1 per cent from 0.1 to 1 per cent had two distinct effects: (a) partial sterilization; (b) chemical action decomposing some of the soil organic matter. The amount of caustic lime necessary to induce specific changes in the flora and fauna of the soil depended largely upon the character of the soil used, and varied from 0.2 to 1 per cent. It was observed that each of the soils, as well as many others examined, appeared to absorb directly a definite amount of caustic lime and until these requirements were fully satisfied, the partial sterilization phenomena (*i. e.* a sudden initial decrease and subsequent increase in the numbers of bacteria, the extinction of the larger forms of protozoa and the inhibition of nitrate production) did not set in. They found that the return in nitrogen, for each increment of lime applied, varied with the character and reaction of the soil and the carbonate content, the average amounting to approximately 1 per cent by weight of the caustic lime applied. The carbonate gave smaller returns, apparently because of its relative inaction on soil organic matter. The pot experiments were comparable with the laboratory data secured, which is summarized in the table on the following page.

This investigation proves without a doubt that caustic lime has an antiseptic value sufficient to induce partial sterilization under certain conditions—but it appears that there has been no definite line of demarcation drawn between the bacterial and chemical activity following the use of lime, thus we are at a loss whether to ascribe the increase in numbers of bacteria following treatment, to the removal of protozoa, or to the increased food supply as a result of the chemical action on organic mat-

ter, for in some cases an increase in bacterial numbers is accompanied by an increase in ammonia production, whereas in other instances there is a decrease in the production of ammonia, and yet again we find an increase in the amount of ammonia accompanied by a decrease in the numbers of bacteria. Thus it is hardly safe to assume that only one limiting factor is operating with such results. Engberding (74) also finds that 0.1 per cent CaO applied to the soil caused an increase in the numbers of bacteria.

EFFECT OF CaO AND CaCO₃ ON PRODUCTION OF NH₃ AND NO₃ (PARTS PER MILLION IN VARIOUS SOILS)

(Table taken from the Report of the British Association for the Advancement of Science, 1913, p. 774)

Soil	% CaO							% CaCO ₃
	.0	0.1	0.2	0.3	0.4	0.5	0.1	1.0
Rothamsted	8	21	40	44	46	54	69	8
Chelsea	31	36	41	49	57	59	75	33
Woburn (acid)	12	29	45	48	54	60	35	49
Craibstone	23	18	27	35	44	49	90	33

Pickering (247, 248, 249) attacked the problems of the action of heat and antiseptics mainly from the agricultural chemist's standpoint. He maintains that the retarding effect of germination produced by heating the soil cannot be explained by an alteration in the bacterial condition of the soil, for the alteration extends progressively at temperatures beyond that sufficient to destroy all bacteria. He further believes that a nitrogenous compound (not of an acid nature) is formed (due to the increase of soluble organic and nitrogenous matter by heating) which is inhibitory towards germination, and is sufficiently stable in solution for an extract of heated soil to affect an unheated soil when it is added to the latter, but that a high temperature and the presence of sufficient moisture, cause it to lose some of its inhibitory properties, probably through oxidation.

At 200° C. the inhibitory substance is formed in greatest quantity, and it diminishes in amount as the temperature is further raised until it disappears entirely at a low red heat. The substance which is inhibitory as regards germination need not necessarily be so as regards plant growth, or it may be destroyed before growth becomes active. Its presence results in an increase of soluble nitrogen in the soil, and this is regarded by Pickering as the chief, if not the sole reason for the increase in growth of non-leguminous plants in heated soil.

As for the treatment of soils with antiseptics, such as CS₂, chloroform, ether, benzene, etc., he finds that the soils undergo chemical change, and that the soluble organic matter is increased, just as when they are heated; likewise they exhibit the same inhibitory effect upon the germination of seeds that heated soils do. Although different antiseptics differ in intensity of action, the inhibitory substance formed, thinks Pickering, is probably the same in all cases (including that formed by heat) for it has the

same effect on the germination of seeds whether produced by antiseptics or heat. He considers that the treatment of soils with antiseptics induces a change equivalent to that obtained by heating the soil from 60° to 75° C., and that this may account for the increased growth of plants. Still Pickering maintains that there is no connection between the fertility of a soil and the extent to which it can be altered by heating, and notes further that soils which are richer and contain most soluble organic matter are slightly less favorable to germination than poorer soils. He found that heated soils behaved toward the starting of the growth of young apple trees as they did toward the germination of seeds, the starting of the growth being considerably delayed; later, however, the trees became very vigorous.

There are several investigators who have applied Russell and Hutchinson's conclusions to the interpretation and practice of sterilization. For example Aitken (1) calls attention to an instance of increased productivity following the heating of the surface soil by a large continued fire. Mann (209) also comments on the practice of burning brush in India, showing that it causes favorable changes in the bacterial flora, renders soluble organic nitrogenous material more available, and improves the physical properties of the soil. Dyer (67) calls attention to the practice followed by large growers of vegetables under glass near London of partially sterilizing their soil by means of steam as giving results on a large scale, confirming the conclusions of Russell and Hutchinson regarding the influence of soil sterilization. Howard (139) explains the beneficial effects of the practice followed in India of exposing the soils to intense heat and light of the hot weather of April and May in the same manner.

Russell (258), commenting on Howard's work suggests the desirability of further investigations to determine to what extent exposure to strong sunlight will bring about partial sterilization and increased productivity of the soil. In a later article Russell and Petherbridge (269) discuss various appliances for partial sterilization by means of heat and the relative efficiency of different antiseptic treatments. Although none of the antiseptics used were as effective as heat, several were found to be superior to toluol which has heretofore been used by the authors. The most effective group of substances included formaldehyde, pyridene, colidene, lutidene, etc. Hiltner (135) also has devised a CS₂ distributor which is safe to use.

Several investigators have recorded increased crop results from the application of sulfur, and it has been suggested that this is a result of partial sterilization. Among those working on sulfur in this connection were: Chancrin and Desriot (41), Pfeiffer and Blank (244), Jonicaud (149), and Russell and Hutchinson (266).

In the past few years several theories regarding partial sterilization have been advanced, a number of which are strongly opposed to those of Russell and Hutchinson. One of the more important of these is the toxin theory held by Fletcher (81, 82, 83), which, however, does not coincide with that of Pickering, for the former contends that heating soils does not produce toxins which delay germination of seeds, but that this delay is caused by decreased imbibition of water from the strong solution in heated soils. He immersed seeds in heated and unheated soils and in extracts of the same soils, and found that at the expiration of 11 hours and 47 hours, respectively, the seeds in the unheated media had absorbed a greater quantity of water.

Fletcher grew plants in a nutrient solution until he considered it to be made toxic by plant excreta. He then steamed it at a pressure of 150 pounds for 2 hours. Plates of an organic substance were thrown down, which did not dissolve on removal of pressure. This substance Fletcher considers the toxic excreta.

The advantage of heating soils, according to Fletcher, does not come from the changing of the bacterial flora or increased soluble matter but from the destruction of the toxin present in unheated soil. He heated soils to 95° and 170° C. and found a better growth on the heated soil than on the control.

Fletcher criticizes Russell and Hutchinson, claiming that toluene renders soil toxins insoluble, and further, that complete sterilization gives an increase crop yield with sorghum over partially sterilized soil.

Another development or variation in the same direction as that of Fletcher is the theory of bacterio-toxins advanced by Greig-Smith (112, 113, 114, 115, 116, 117, 118, 119), who holds that there are bacterio-toxins present in soils; for by the extraction with water of soils of varying fertility and filtering through porcelain, he obtained a solution which either destroyed or inhibited bacterial growth; these extracts upon boiling for an hour behaved as nutritive solutions. He notes that soil bacteria exhibit a certain degree of immunity towards their own toxins, consequently, he employed a pure culture of *Bacillus prodigiosus*.

Two hundred gm. of poor sandy soil were treated with 200 c.c. of saline for an hour, the extract being heated at 94° (C. probably) for an hour. Two days afterward it was boiled for an hour. The extracts were infected and incubated over night at 30°. In the morning the numbers of bacteria were determined as follows:

1000 Bacteria became:

1. Saline extract of poor soil	0
2. Saline extract of poor soil heated at 94°	2,000
3. Control	117,000
4. Extract (1 above) 2 days after	73
5. Extract (1 above) 2 days after, boiled	3,250,000
6. Control	70,000

The experimenter concludes that a toxin is contained in soil, and is soluble in dilute saline, it is partially destroyed at 94° and rapidly decays in aqueous solution; boiling either converts it into a nutrient, or by destroying the toxin, enables the nutrients dissolved in the saline to act. Thus, heating a soil destroys the bacterio-toxin, which accounts for enhanced fertility.

The toxin is destroyed by sunlight, as shown by exposing a portion of the soil to the sun for 12 hours while another portion beside it was protected. The extracts from these soils were seeded with *B. Prodigiosus* and incubated overnight at 30°, with the following results:

	1000 Bacteria became:
Soil exposed to light	227,000
Soil protected from light	11
Soil in laboratory	9

The power of the toxin is not diminished by salts, such as sodium chloride, potassium sulfate, or magnesium sulfate (says the author without furnishing any data), for the toxic effect of extracts of soil made with 0.5 per cent solutions of these was very pronounced.

The effect of disinfectants is to dissolve the *agricere* (or soil wax) as well as to kill off the less resistant bacteria, thus enabling the surviving bacteria to obtain a greater food supply, in consequence of which there is a greater availability of the organic nutritive matter of the soil.

Bottomley (26) also considers the effects of bacterio-toxins in the soil. Greig-Smith (118), in a later communication, finds that the action of toluene upon the soil protozoa of the soil depends upon the moisture-content of the soil. When less than one-tenth of the water-holding capacity of the soil is present, certain members of the ciliates, *amœbæ*, and flagellates may not be destroyed, when amounts up to 20 per cent of toluene are added to the soil. If more than one-tenth of the water-holding capacity is present, the ciliates are destroyed by 1 or 2 per cent of toluene, while the action upon the *amœbæ* and flagellates is irregular. Conditions which destroy the sulfur-oxidizing bacteria also destroy the ciliates.

Jensen (151), however, reports that results obtained from treatment of soils by heating and antiseptic treatment made no appreciable difference in the solubility of the mineral fertilizing ingredients in acids, and fails to support Greig-Smith's (*loc. cit.*) theory of water-proofing the soil particles by *agricere*—while confirming Russell and Hutchinson's conclusions.

Regarding "soil-sickness" or "fatigue," a phenomenon which Russell and Hutchinson explain as partly due to the presence of protozoa, we find that Loew (202, 203) and Fawcett (77), working with some "sick soils" where conditions were the worst, noted the complete absence of

protozoa. Samples of these soils were disinfected with heavy applications of CS_2 , but from the results of the study it appears that any benefit that is derived from the disinfection of the soils cannot be attributed to the destruction of protozoa.

Jachevski (146) also states that soil fatigue is frequently attributed to wrong causes, citing cases where the effects were due to fungi (naming the species). Disinfection with CS_2 , etc., is recommended as a treatment for alleviating this condition.

Bolley (22, 23, 24, 25) also believes that fungi have more to do with the increase in crop growth after sterilization than is ordinarily supposed.

Bokorny (21) mentions CS_2 and toluol among numerous other chemical substances injurious to fungi; and Kober (167) confirms the conclusions of Muth (227) concerning the antiseptic value of CS_2 . Emmerich (72) obtained successful results with the use of CS_2 and carbolineum for cleansing soils infected with nematodes. Stone (296) reports that soils rich in organic matter sterilized at 180° to 212° F. give good greenhouse results; and claims that the good effect is due to chemical action and aeration.

The sterilization of the soil has been the concern of both the practical agriculturist and the scientist. Pickering's (loc. cit.) consideration of the chemical effects of sterilization was especially significant, consequently, any additional information bearing upon this line of inquiry deserves attention. Lyon and Bizzell (205) determined the effect of sterilizing soils by steam on the water-soluble matter, and found that steaming the soil at 2 atmospheres reduces the nitrates to nitrites and ammonia, but that most of the ammonia is formed from organic nitrogen. On standing uncropped the steamed soil steadily decreased in its content of soluble matter and both ammonification and nitrification were stopped. The authors conclude that the time of recovery varies with productiveness, and that the injurious substances produced by steaming and recovery from these injurious effects is hastened by the growth of plants (wheat). An infusion of unsterilized soil added to the steamed soil increased the germination of seeds and early growth, but retarded later growth, and hastened the disappearance of total water-soluble matter, while it did not increase ammonification or nitrification.

From this and later work (206) it is to be concluded that toxic matter controls the productivity in steamed soils, and that the condition of the organic matter before steaming influences toxicity. Furthermore, the fact that the soil is unable to rid itself of toxic material formed by steaming, indicates that a similar condition causes sterility in the field.

Schreiner and his associates (277, 278, 279) have presented an interesting phase of this problem in their investigation on the chemistry of steam-heated soils. They conclude from their results obtained when working with Elkton silt loam and Sassafras silt loam (10 lbs. of which

was heated at 30 lbs. pressure for 3 hours at 135° C. and extracted with 2 per cent NaOH) that the heating of soils in this manner causes an increase in the water-soluble constituents of the soil as well as an increase in acidity. At the same time, ammonia and amines are formed, and there is an increase in all the constituents isolated from the unheated soil except nucleic acid. By the process of heating, xanthine, hypoxanthine, guanine, cytosine, and arginine are discovered, whereas these did not exist in the soil previous to treatment. The authors regard these compounds as decomposition products of nucleic acid and protein material and that they are all beneficial to plant growth.

Guanine is a constituent of soil organic matter (reported for the first time). Dihydroxystearic acid increases when present originally, and is produced by the heating process, if not present previously. It is harmful to plant growth. Thus the isolation of both beneficial and harmful compounds produced in soils by heating bears out the cultural tests of other investigators. Cultural tests in these soils and their extracts showed that heated soils gave a poorer plant growth than unheated soil. Although the majority of compounds, isolated by these authors, must be classed as beneficial, nevertheless, the harmful compounds formed at the same time more than overbalance the good effects, and they insist that not until these harmful compounds are eliminated or diminished can the full benefits of heating the soil be demonstrated. They maintain, furthermore, that in soils there is a balance between the beneficial and harmful factors, and soil fertility or infertility represents the resultant of these two groups. This balance is influenced by cultural treatment, fertilizers, liming, crop growth, crop rotations, etc., as well as by steaming.

Schreiner concludes from his results that although the soils studied have received the same kind of organic matter and the same farm treatment, they have been subjected to different biochemical factors, which result in a difference in their organic matter as well as a difference in their fertility.

Some further significant biochemical studies on the effect of subjecting soils to heat (in this case dry) were those of Seaver and Clark (283), who sterilized soils at 90°, 120°, 150° and 170° C. with the following results:

Soil	Total Solid Matter %	Organic Matter %	Inorganic Matter %	Total N %
Unheated	0.030	0.020	0.010	0.0012
Heat to 90° C.	0.061	0.045	0.016	0.0016
Heat to 120° C.	0.117	0.092	0.025	0.0036
Heat to 150° C.	0.219	0.177	0.042	0.0092
Heat to 170° C.	0.275	0.184	0.091	0.0112

They conclude that an increase in the large amount of soluble matter goes hand in hand with the temperature to which the soil is subjected

Heating to 120° C. for 10 hours gave little increase over 2 hours' heating. Testing the extract of heated soil, they find that it consists chiefly of a carbohydrate-like substance probably derived from the cellulose remains of previous plant growth upon the soil. It reveals the properties of sugars and organic acids.

They grew oats and lupines at the temperatures noted above, and observed an increase of growth to 120° C. and above that a retardation, due, they believe, to the fact that the plant cannot absorb as much soluble matter as is present, rather than the existence of any toxin. It is to be noted that the growth of fungi was increased by heating the soil.

We have observed that a number of investigators have sought to affirm or deny the validity of Russell and Hutchinson's conclusions regarding the protozoan theory of fertility, and it is striking that with comparatively few exceptions, most of these scientists have failed to make any direct examination of soil protozoa, basing their conclusions entirely on the indirect results obtained by sterilization of soil, extracts, etc. Thus it is evident, that no matter how sound their work appears, it does not carry with it that note of finality which is eminently desirable for a just criticism of the work of Russell and Hutchinson. It is true, nevertheless, that as a group they have attached sufficient importance to the rôle which bacteria play in this complicated problem, in fact their principal emphasis seems to fall (on the experimental side at any rate) on bacteria rather than protozoa.

A careful piece of experimentation is that of Fred (89) who used loam soil (mixed with sand) and found that 2 per cent CS₂ has little effect upon the moisture content of the soil. With varying percentages of ether (together with 2 per cent of sugar) in the soil he finds an initial depression in bacterial numbers followed by a considerable increase in 8 hours, 4 per cent giving the maximum count. Antiseptics in sufficiently dilute solutions were found to stimulate bacterial development [including the yeasts, *azotobacter*, and ammonifying, putrefactive, and denitrifying organisms, contrary to Hiltner's (*loc. cit.*) observations], and further nitrogen-fixation is increased.

In order to test the validity of Russell and Hutchinson's conclusion that the absence of protozoa (by treatment with toluene) is responsible for increased production of ammonia, Fred, using ether instead of toluene, subjected one series of flasks containing compost soil to 100° C. moist heat for an hour and used a similar series, unheated, as a check. All the flasks received 0.2 per cent ammonium sulfate—some of the flasks received 2 per cent and 5 per cent ether. In order to obtain vigorous nitrification 170 c.c. of amoebæ-free extract was inoculated into all the flasks. (This was prepared by leaching 2 kg. of compost soil and 4 liters of sterile water and filtering through filter paper; the microscopic examination revealing the presence of no amoebæ).

The analyses for nitrate nitrogen were made at the beginning of the experiments and at the end of 100 and 150 days, respectively, the results showed that heating the soil to remove amoebæ did not have a beneficial effect upon nitrate formation, contrary to Russell and Hutchinson's work—although the addition of a small amount of ether increased nitrification in the flasks containing amoebæ, and had the opposite effect in the soil free from amoebæ. This, the author believes, may be accounted for by the stimulating effect upon the nitrifying bacteria since the heated soil not treated with ether showed no such increase.

Fred concludes (in addition to the above-mentioned observations) that ether and CS_2 cause an increased fixation of nitrogen in pure cultures of *Azotobacter*. The development of denitrifying organisms is hindered for only a short time, because of treatment with antiseptics. Both *Azotobacter* and denitrifying organisms are insignificant, in a normal soil. Nitrification is at first inhibited and later accelerated by antiseptics, while toxins remain unaffected by treatment. CS_2 and ether cause an increase in crop yield under sterile conditions.

He holds that the increased growth of plants following the use of antiseptics in the soil depends essentially upon the stimulation to the plant itself, in combination with a similar effect on the lower organisms.

Fred's work is highly suggestive, but the determination of nitrogen produced is in the form of nitrates alone, and no data are set forth concerning ammonia. That this might affect his results is evident when one takes into consideration the fact that most investigators have proven that nitrification is depressed by antiseptics.

Furthermore, like many other experimenters he does not consider the possibility of protozoa cysts passing through the filter paper in the preparation of "amoebæ-free extract." And we have found in our experimental work that cysts do pass through several thicknesses of high-grade filter paper.

In much the same manner, Gainey (96) concludes from investigations relative to the effect of toluol and CS_2 upon the micro-flora and fauna of the soil, that: (a) small quantities of CS_2 , toluol, and chloroform, such as have been used practically and experimentally, when applied to soils studied, exert a stimulative rather than a diminishing effect upon the total number of bacteria present; (b) an application of such quantities of toluol and CS_2 does not have an appreciable effect upon the number of types of protozoa present; (c) a very marked increase in yield may be noted following such an application when no evident change occurs in the total number of bacteria present; and (d) these conclusions together with the work of Koch, Egorow, Goody, Fred and others, make Russell and Hutchinson's theory untenable.

Among other investigators who fail to confirm Russell and Hutchinson's conclusions are Laidlaw and Price (194) who state the following

conclusions (and do not substantiate them with any experimental evidence).

(a) The increased fertility of partially sterilized soils is due to the new bacterial flora being a more active decomposing agent than the original one and causing an increase in ammonia. (b) Protozoa being killed off by treatment serve as food for the new bacterial flora as well as food for the plant in the form of ammonia, and since some of these large organisms devour bacteria, their destruction allows a rapid development of the new bacterial growth to take place.

Some further work which is manifestly incomplete is that of Lodge and Smith (201) who investigated the influence of soil decoctions from sterilized and unsterilized soils upon bacterial growth, and found that sterilization accelerated bacterial growth on soils rich in organic matter, but failed to isolate protozoa from their soil, which fact is a striking one, and rather hard to accept, as Russell suggests. It may be further stated that it barely seems justifiable for the authors to base any such generalization upon the use of but two soils.

Greig-Smith (116), continuing his previous investigations, endeavored to test the action of the soil protozoa, (a) by purposely adding them to the soil, and (b) by using the extracts of raw soil as was done by Russell and Hutchinson, taking care to use soil that had not been overheated and to have controls of unfiltered soil extracts to compare with the filtered, presumably protozoa-free extracts. He concludes from his experiments as a whole "that Russell's contention cannot be sustained; the protozoa have little or no action in limiting the number of soil bacteria."

Greig-Smith found that the larger ciliates, such as *Colpoda cucullus*, were not destroyed when comparatively large amounts of volatile disinfectant were added to the soil. Upon adding suspensions of protozoa, there was no evidence of any limitation in the numbers of soil bacteria. Any enhanced effect was due to the addition of bacteria contained in the suspension. The filtration of a soil extract had no influence, beyond that of removing some of the bacteria in suspension. Any phagocytic tendencies that soil protozoa possess, he finds, have no influence in limiting the numbers of bacteria in the soil.

So far as the growth of bacteria was concerned, the effect of heat was of a different character from that of a volatile antiseptic. Thus he infers that toxins and nutrients of the soil are alone concerned with the changes that occur when soils are heated or treated with volatile disinfectants.

In his latest contributions to the subject, Greig-Smith (119, 120) advances a theory to account for increased bacterial activity following the application of antiseptics such as chloroform. He finds that with the methods now in vogue all of the antiseptic applied is not removed, but that traces remain which are sufficient in amount to stimulate bacterial development.

Lipman, Blair, Owen and McLean (199), reporting some experiments relating to the possible influences of protozoa upon ammonification in the soil, used soil sterilized at 1.5 atmospheres in the autoclave, and found that pasteurization does not increase the ammonifying power of the soil infusion, and that sterilization decreases the ammonifying power of the soil. Owing to some contradictory results and the fact that the experiment was of very short duration as compared with that of Russell and Hutchinson (as is indicated by them), it is not conclusive in pointing out any fallacies in the latter's conclusions.

Some investigators have laid considerable emphasis on the bacterial activities in sterilized and unsterilized soils. Lemmermann, Fischer, Kappen and Blanck (196) find that sterilization depresses nitrification and does not increase ammonia production markedly.

Vogel (313a) points out the importance of the denitrifying organisms in sterilized soils.

Greaves and Anderson (110) find that arsenic in certain concentrations, stimulates the nitrogen-fixing power of various soils.

Jamieson (148), however, finds that increased productiveness following the heating of soil or treatment with substances inimical to plant life is "due simply to the riddance in varied degree of the varied forms of animal life in soil that prey on plants" and not to any influence on bacterial activity in the soil.

A recent paper on partial sterilization to come to our attention is that of Buddin (33). In an attempt to test a variety of substances having the power partially to sterilize the soil, Buddin has employed a number of volatile and non-volatile antiseptics on two kinds of soil (type not stated—and moisture content varying for no given reason). He finds that the characteristics of true partial sterilization (obtained only with volatile or removable antiseptics), namely, an initial decrease in numbers of bacteria followed by a large sustained rise, the killing of protozoa and nitrifying organisms, and an initial increase in ammonia content followed by a considerable increase in the rate of production of ammonia (for which he has presented no individual data), are common to a large number of antiseptics.

Having failed to find the non-volatile substance which might prove of commercial value, the author makes a few generalizations (and little qualitative bacterial work is recorded), such as: "It seems to be a general rule that a simple flora can attain extraordinarily high numbers, while a complex flora after normal partial sterilization does not attain to higher numbers than the comparatively low level of about 5 times those in the untreated."

Again, concerning the statement that "no increase in dose causes any change in the results obtained once true partial sterilization has set in with any particular chemical"—the results appear to be contradictory.

Doses of benzene, for example, beyond the partial sterilization point, result in an increase not only of bacteria but ammonia and nitrate as well.

(Taken from Jour. Agr. Sci., v. 6 (1914), p. 426, Table I)

	Gm. of Benzene added per kg. of Dry Soil	Bacteria Present Millions per gm. of Soil			NH ₃ and NO ₃ P. P. M. Dry Soil		Effect on Protozoa
		At Start	After 25 Days	After 47 Days	At Start	After 26 Days	
M/100..	0.78	10	30	23	30	41.5	C. A. M. (M) (M)
M/50 ..	1.56	8	78	85	30	60.5	
M/5 ..	15.60	6	90	92	27	63.5	

"It will be noticed," says Buddin, "that the partial sterilization point occurs somewhere between the M/100 and M/50 dose." Yet we also notice a considerable increase in bacterial numbers and some increase in the nitrogen, contrary to the author's explicit conclusion.

We have endeavored in the preceding pages to present a review of the literature dealing with partial and total sterilization by heat or antiseptics, and in some cases it was deemed advisable to offer certain criticisms of the investigations under consideration; yet these were sketchy in character in lieu of the fact that the major portion of the experimental work now in progress has as yet not furnished sufficient results to warrant more convincing argumentation.

Russell and Hutchinson have considered certain objections to their work, and it is only fitting in concluding this portion of the article, to state their views on these more important criticisms. It is to be remembered that they emphatically maintain that the organic matter of the soil suffers decomposition on heating to high temperatures, thereby changing the soil as a medium for the growth of organisms. From their point of view Seaver and Clark, Fred, and Lipman (*loc. cit.*) stand open to criticism on this score. They discard the theory of a change in soil colloids being a satisfactory explanation of all the phenomena attending partial sterilization, on the grounds that it cannot be "reconciled with the active nature of the factor," and that it involves contradictions when untreated soil is added to treated soil, and vice versa.

The bacterio-toxin hypothesis, they maintain, does not account for the results, "for the depression produced by the introduction of the untreated soil ought to come into operation at once if it were caused by a toxin." Further, "the water-extract of an untreated soil has no toxic effect when added to a tolued soil and not infrequently causes an increase in bacterial numbers because it itself carries bacteria." The above statements invalidate the work of Grieg-Smith and Bottomley (*loc. cit.*).

In a lecture on microorganisms of the soil, Moore (222) made some caustic allusions to Russell and Hutchinson's work which certainly demand the support of experimental evidence (the author mentioning

various results, which so far as we have been able to ascertain have remained unpublished). "I could show," he says, "as the result of tests over a wide field, that the number of protozoa, including flagellates, ciliates, and rhizopods, existing in the soil 3 days after treatment with various per cents of toluol, carbon bisulfid, etc., may equal or even exceed the number originally present. The one fact which does seem to be fairly well established is that the temporary removal from the soil of the protozoa has but little bearing on the problem." To which Russell (259) replies: "We should not have lived in vain if we had merely been the humble instruments by which such a proposition was established, but again we are not satisfied as to the evidence."

SOIL PROTOZOOLOGY

Having thus reviewed the work on sterilization, we are now enabled to obtain a perspective of that field of investigation; and in so doing view the birth of a new branch of soil biology, namely, soil protozoology. The observer will readily admit that until the last few years that field of science has remained comparatively unknown. It needed the impetus of Russell and Hutchinson's work to awaken it to active life, and it is for this reason that we may justifiably regard soil protozoology as an outgrowth of the investigations in partial sterilization. It is not to be presumed that experimentation in soil fertility was entirely responsible for the creation of this science, even though it furnished the major stimulus. The general biologist has long been familiar with this lowest form of animal life, has studied and experimented upon it—but he has had no occasion to consider it preeminently as an organism to be isolated from the soil. It has long held an important place in the fields of human and animal pathogenesis, and its morphology, as well as its life-habits, have been closely studied. What then, one might well ask, has the protozoologist given the soils investigator as a heritage?

Protozoa are "unicellular organisms, containing chromatin or nuclear substance, which usually forms nuclei readily distinguishable from the protoplasmic body, being either naked at the surface or enclosed by a cell-membrane." Organs for locomotion and the capture and assimilation of food are usually present, although they may be absent entirely. Reproduction is effected by some form of fission, and in the vast majority of protozoa, if not in all, a process of conjugation or syngamy occurs at some period in the life-cycle, the essential feature of the process being fusion of nuclear matter from distinct individuals.

Under adverse conditions most protozoa are capable of going into encystment, a state analogous to the spore-forming stage of bacteria; the ectocyst may be of a calcareous or even siliceous character.

For general treatises on protozoa the student may turn to any number of excellent productions coming from Bütschli (35), Döflein (64), Minchin (220), and others (14, 50, 68, 75, 158, 162, 178).

Doflein is the only author mentioned who has given soil protozoa any serious consideration, and he has presented some exceedingly useful information.

Methods in Soil Protozoology

The foundation of a science, it might well be stated, rests largely upon its methods; consequently, we can expect no striking development of soil protozoology for the reason that little work has been done on this phase of the problem—and that which has been done is in a very chaotic and unstandardized state. We might even go so far as to point out that a general investigation and establishment of accurate methods is at present the primary requisite for any sound work on the problems of soil protozoa in relation to soil fertility. However, it is of interest to note the work which has already been done in this direction, and it is our purpose to present a review of the material to date, considering soil protozoology under the topics of (a) Methods, and (b) Activities.

1. Media

As a general medium for the isolation of protozoa a 1 per cent hay infusion seems to be most widely used. Zaubitzer (335) (who gives a good review of methods); Russell and Hutchinson (loc. cit.), and Goodey (104), who made the infusion slightly alkaline to litmus and added egg albumen and 0.75 per cent NaCl, as well as Fine (80) and Woodruff (332, 333, 334), have all employed this medium.

In a few cases bacterial solutions have been employed for the cultivation of soil protozoa, as for example, Beijerinck (13), Wolff (330), Emmerich, Graf zu Leiningen and Loew (72) have noted the occurrence of protozoa in association with *Azotobacter*.

Cunningham and Löhns (54), in an admirable paper, have made a survey of the following bacterial solutions: (1) ammonifying solutions, i. e. (a) 1 per cent bloodmeal and 0.05 per cent K_2HPO_4 in tap water; (b) cornmeal and fleshmeal solutions (which gave very similar results to those obtained with bloodmeal, fleshmeal giving a rather better development than the other two); (c) 1 per cent solution and 0.05 per cent K_2HPO_4 (which proved too concentrated); (2) Nitrifying solution of Windgradsky-Omelianski, using 0.1 per cent $(NH_4)_2SO_4$; (3) Denitrifying solution (Giltay); (4) *Azotobacter* media (a) 1 per cent mannite and 0.05 per cent K_2HPO_4 in soil extract; (b) 2 per cent calcium malate and 0.05 per cent K_2HPO_4 in tap water; and (c) a number of mannite solutions where butyric acid bacteria replaced *Azotobacter* which were unfavorable to the growth of protozoa; (5) urea solution (5 per cent) and 0.05 per cent K_2HPO_4 in soil extract, which showed no protozoa; (6) cyanamide solution, 0.2 gm. cyanamide, 0.05 per cent K_2HPO_4 , 0.01 gm. asparagin, 0.01 gm. glucose, in which practically no protozoa were found; (7) solution for cellulose decomposition (Omelianski); (8) (a) 1 per cent

mannite, 0.05 per cent K_2HPO_4 , chalk and *Bacillus fluorescens*; (b) cellulose organisms on filter paper and K_2HPO_4 and $MgNH_4PO_4$; (c) soil extract and 0.05 gm. K_2HPO_4 ; (d) soil extract and K_2HPO_4 agar plates.

The striking feature of the results brought out by the survey of the media is the close connection between the development of the protozoa and that of the bacteria, although the protozoal activity lags slightly behind that of the bacteria. It has been found also that there is a general sequence in the development of the various groups of protozoa, flagellates appearing first, followed almost immediately by ciliates, and later by the amœbæ. The most satisfactory media reviewed above were: (a) bloodmeal solution at 22° C. for flagellates in the early stages, later for ciliates; (b) bloodmeal solution at 30° C. for ciliates; (c) Giltay solution at 22° C. for ciliates; (d) soil extract and K_2HPO_4 at 22° C. for all three types, but chiefly for flagellates and ciliates; (e) mannite solution and chalk inoculated with *B. fluorescens* incubated at 22° C. for ciliates and amœbæ.

Kopeloff, Lint and Coleman (183, 184, 185, 186) have reported studies with various media for the development of protozoa. It was found that 10 per cent hay infusion with and without albumen was most desirable. Among the new methods devised by them for the study of protozoa in relation to soils is an adaptation of the blood-counting apparatus (Blutkörperzählapparat) for the counting of protozoa, an agar plate method for separating fungi from bacteria and protozoa, and a modification of the filter-paper method for the separation of different kinds of protozoa.

Killer (163) also reviews bacterial solutions, employing the solution for peptone decomposition (100 c.c. distilled water and 1 gm. peptone); Omelianski's nitrification; Giltay's denitrification; Hiltner's denitrification (100 c.c. distilled water, 1 gm. peptone and 1 gm. $NaNO_3$); solution for nitrogen assimilation (100 c.c. distilled water, 2 gm. mannite and 0.05 gm. K_2HPO_4); solution for urea decomposition (100 c.c. distilled water, 1 gm. peptone and 10 gm. urea); Zumstein solution for culture of flagellates (0.5 gm. peptone, 0.5 gm. grape sugar, 0.2 gm. citric acid, 0.02 gm. $MgSO_4$, 0.05 gm. K_2HPO_4 and 100 c.c. H_2O).

The above solutions were used undiluted, 5 and 10 times diluted, and inoculated with fallow soil, to be examined every 10 and 20 days. He found that the protozoa developed best in the Giltay denitrifying solution, but that the chemical constitution as well as the concentration of the media has a definite influence on developing the numbers and kind of protozoa developed, as is shown by the detailed record of the protozoa appearing in the solutions used. While Cunningham and Löhnis found few, if any, Killer reports amœbæ in large numbers in the solution for the culture of flagellates. Solid media (plating method) has been employed rather extensively for the cultivation of amœbæ, Killer recom-

mending Beijerinck's (13) addition of 0.20 per cent $\text{NH}_4\text{NaHPO}_4 \cdot 4\text{H}_2\text{O}$ and 0.5 per cent of KCl to agar-agar. This method was also used by Tischutkin (309). Celli and Fioca (39, 40) were the first to make pure cultures of amoebæ from a 5 per cent water solution of *Fucus crispus* with and without the addition of bouillon on alkaline potato, etc. They report amoebæ, *guttula*, *oblonga*, *undulans*, *arborescens*, etc. Miller (219) corroborated this work using neutral bouillon 2 to 4 parts in 100 parts of water with 0.5 per cent glycerine and a hay infusion with 0.5 per cent grape or milk sugar. Behla (9), Casagrandi and Barbagallo (37), and Sharding (271) used hay agar with success. Berliner (15) used an agar medium of 90 per cent tap water, 10 per cent bouillon, and 5 per cent agar, while Martin and Lewin (213) recommends a horse-dung agar prepared by boiling three lumps of horse-dung in 500 c.c. water for $1\frac{1}{4}$ hours, filtering through a cloth and adding 6 gm. agar. They add a small amount of water to the culture plates to obtain strong growth.

Doflein (64) in his valuable book describes the following medium as being especially adapted to the growth of *Euglena*: 0.5 gm. peptone, 0.9 gm. grape sugar, 0.2 gm. citric acid, 0.02 gm. $\text{Mg SO}_4 \cdot 7\text{H}_2\text{O}$, 0.05 gm. K_2HPO_4 .

It is evident from the foregoing review that no medium was found to be perfectly satisfactory, even though several of those mentioned were found to be successful. Suffice it to say that there is a vital necessity for an intensive, as well as extensive, survey of culture media for the growth of protozoa.

2. Staining

The staining methods in vogue for soil protozoa have been carried over for the most part, from the laboratory of the general protozoologist, consequently there has been no particular reason for the former to go very deeply into such work. Any of the standard textbooks on protozoa previously mentioned may be consulted to advantage, and Lee's "Vade Mecum" is an indispensable guide to the preparation of stains.

Goodey (103) has made the most exhaustive study of staining methods of any of the soil protozoologists. To kill an organism he exposes it to osmic vapor, and for quick or nuclear staining, adds a saturated solution of methyl green in 1 per cent acetic acid, and then paints the edge of the cover glass with the hot wick of a candle to prevent evaporation. He has also employed the following well-known stains: picric acid [also used by Martin and Lewin (213)], iodine green in a saturated aqueous solution and 1 per cent acetic acid, neutral red for staining food vacuoles, safranin, gentian violet, eosin, carbol fuchsin, Heidenhain's and Delafield's iron haematoxylin (which he finds to be the best for staining cyst membranes, etc.).

Zaubitzer (335) has also employed a number of these stains together with eosin and thionin, methylene blue and thionin, etc. Sun (306) uses "Alaunkarmin."

Others to report on stains were Tsujitani (310), Roemer (254), Janowski (150), Schaudinn (272).

For quick work with film preparations, picric acid is generally recommended, and we have also obtained good results with aceto-carmin. For permanent slides, iron haematoxylin is most efficient.

3. Counting

Again a great difficulty is encountered in the technique of counting because until recently there has been no entirely satisfactory method. Four have been employed: (a) direct counting of a drop by means of a microscope, which is the most common practice; (b) the dilution method as used in the counting of bacteria, suggested by Rahn (251), who places 1 c.c. of the solution in sterile media and dilutes 1:10, 1:100, 1:1000, 1:10,000 and determines the dilution above which the protozoa are not found by examination at periodic intervals, and below which they do exist—naturally, only a rough approximation—a method which has been employed to some extent; (c) agar-plating method used by Killer (163) and others, the principal difficulty being in differentiating the protozoa from bacteria and organic matter; (d) by counting per standard loop, a method devised by Müller (225) for bacteria and other microscopic organisms. He further recommends saponin for suppressing protozoa in bacterial cultures, which was found to be unsuccessful by Cunningham (52, 53).

Kopeloff, Lint and Coleman (183) have recently adapted the Blutkörperzählapparat, whereby the protozoa may be counted accurately. Statkewitsch (291) advises a slimy colloidal medium for reducing the motility of protozoa, as for example, *semen psylli*, *semen cydoniae*, *gumme tragacanthae*, etc.

Biffi and Razzeto (20) are the only authors to describe in any detail the separation of protozoa from bacteria other than the method of having protozoa feed on dead bacteria as described by Jordan (157). Russell and Hutchinson (265, 266) employed cotton-wool, and others have mentioned filtration of one kind or another, but these investigators found that the quality of the filter used, as well as the nature of the cell, the quality of the suspension solution, the temperature and length of filtration all determined the passage of organisms through the filter. They found further that all water-protozoa pass "amikrobischen" filters, that the large protozoa passed through most permeable filters, that the small protozoa passed through slightly permeable filters, and that the amœbæ passed through all. An interesting observation, too, was that protozoa change their shape, growing longer and narrower to pass through the canals of a filter. Russell and Hutchinson, Jones (156) and Koch (175) note that the centrifuge may be employed as a means for separating different kinds of protozoa. Kopeloff, Lint and Coleman (186) have separated different types of protozoa by the filter paper method.

Protozoa and Their Activities

The morphology, physiology and life-history of soil protozoa have been the subject of some investigation, although again the greater part of such information has come from the general protozoologist. Owing to the limited material available for review from this point on it becomes necessary to consider the work of individuals rather than group them with reference to that particular phase which interested them most.

For a fairly complete list of the species of soil protozoa one may turn to Wolff (329), who lists the following as being among these predominant:

SARCODINA: *Hyalodiscus limax*, *H. guttula*, *amaba terricola*, *arcella*, *vulgaris*, *diffugia*, *constricta*, *gromia*.

FLAGELLATA: *Monas guttula*, *M. vivipara*, *salpingoeca*, *S. convullaria*, *Bodo ovatus*, *B. saltans*, *B. angustus*, *B. caudatus*, *Phyllonutius undulans*, *B. euromonas jaculans*, *Euglena viridis*, *Polytoma uella*, *chlamydomonas monadina*.

CILIATA: *Nassula elegans*, *Glaucoma scintillans*, *Colpidium colpoda*, *C. Cucullus*, *Balanthophorus minutus*.

In all probability Goodey (103) has contributed more to our knowledge of soil protozoa, especially as regards a comparison of the living and cyst stages, than any other investigator. He gives a systematic classification of soil protozoa, observing that *Colpoda cucullus* and *Colpoda steinii* are most common. Russell and Hutchinson likewise found that the former played an exceedingly significant rôle in the soil. In an attempt to separate the cysts from free living forms, Jennings' method of thermotaxis was employed, and found wanting. A method of galvanotaxis, however, with a continuous current between two non-polarizing electrodes caused the organisms to collect at the cathode. The solution from the incubated infusion was examined, and it was found that active ciliated protozoa developed $4\frac{1}{2}$ hours after inoculation where large quantities of soil had been used.

The method of excystation was observed in hanging drop and it was found that the ectocyst ruptures, the organism within rotates, the contractile vacuole pulsates, and finally the endocyst swells and breaks. The time necessary for this process may be as low as $1\frac{1}{2}$ hours after incubation, but is usually from 2 to 4 hours in duration. No food vacuoles appear in the organism immediately after excystation.

Goodey reaches the following conclusions. (a) Ciliates in soil cultures are due to active protozoa and cysts. (b) The incubation period for the first protozoa to appear from soil culture and the time required for the earliest *Colpoda* to come out of its cyst, are the same, indicating that the original condition is similar, i. e., one of encystment. (c) The first *Colpoda* occurring from the soil is similar to those from cysts. Had they been feeding, the living protozoa would have had food vacuoles; therefore, he concludes that they must be present in the soil as resting cysts.

(d) Under conditions favorable to bacteria and protozoa, the first protozoa come from the resting cysts. The first *Colpoda* to appear from a soil to which they had been added, did so only after the time necessary for emergence from cysts had elapsed, and further they resembled the encysted form, showing that the free living forms had encysted. Thus Goodey maintains that ciliated protozoa exists in the soil only in the encysted condition and are not factors limiting bacterial activity.

This was corroborated by a second investigation (105), where some soils stored in bottles since 1846, and 1870, were examined for partial sterilization phenomena. No factor limiting bacterial activity was revealed in the 1846 soil. The 1870 soil, however, contained amœbæ and flagellates and exhibited the usual phenomena accompanying partial sterilization.

In some later work on the encystation of *Colpoda cucullus* from its resting cysts and the nature and properties of the cell membrane Goodey (104, 106) reports the effects of temperature and reaction on resting cysts, and observed that at 40° C. no excystation took place after several hours. At 30° C. many active forms were discovered after 1 hour; at 25° C. a few active forms were obtained after 1 hour and 17 minutes, and at 20° C. a few active forms were observed after 2 hours and 12 minutes.

Using different percentages of NaOH, starting at 0.01 per cent and progressing by increments of 0.01 per cent up to 0.2 per cent, he finds excystation rapid and free in all cultures below 0.15 per cent NaOH; the critical strength of NaOH which inhibited excystation was 0.18 per cent. With HCl, increments of 0.001 per cent were used from 0.001 per cent up to 0.01 per cent, and then of 0.01 per cent up to 0.10 per cent, the latter was found to be the critical point. Thus excystation, he concludes, can take place within wide limits in an alkaline medium containing 0.18 or 0.19 per cent NaOH, and in the presence of 0.09 per cent HCl.

A variety of tests on the nature and properties of the cyst membrane are summarized as follows. The cyst membranes of *Colpoda cucullus* consist of an outer ectocyst and an inner endocyst. The former is insoluble in strong acids; no reaction is obtained with iodine and H_2SO_4 , and iron haemotoxylin is the only stain which is successful. It is insoluble in alcohol and ether, and is only dissolved by 20 per cent NaOH. The endocyst is composed of a transparent substance insoluble in cold and hot water, strong acids and fat solvents. It is soluble in 4 per cent NaOH at 30° C. and gives no reaction with iodine or with H_2SO_4 , but is completely digested by diastase and ptyalin at 40° C. This secretion is named "cystase." Thus, he concludes, the endocyst of *Colpoda*, etc., is composed of a carbohydrate of unusual character named cystose, the enzyme secreted being cystase.

Goodey (107) in a subsequent publication confirms his contention that the ciliated protozoa are present in the soil in an encysted condition

and, therefore, cannot function as a factor limiting bacterial activity. Russell (261) controverts this conclusion by showing that the organisms which Goodey employed by using hay infusion with soil were not identical with the normal soil fauna.

Rhumbler (252) gives a very lucid account of the different cyst formations of *Colpoda cucullus*, which is accompanied by suggestive illustrations and a helpful bibliography.

Woker and Pecker (328) report the influence of blood serum, and salt solutions upon the cysts of *Colpoda cucullus*. Meunier (217) found that encysted *Colpoda* were killed by a temperature of 100° C. Zaubitzer (335) found 15° to 20° C. the optimum temperature for the excystation of amœbæ.

As regards the life cycle of protozoa, Statkewitch (291) finds that it depends mainly upon nutrition, and that decomposition products were responsible for mortality. Mechanical stirring, removal of decomposition products, neutralization with Na_2CO_3 and the addition of salts like $\text{Ca}_3(\text{PO}_4)_2$, etc., were found to prolong life. The life cycle of amœbæ has been the subject of investigation by: Alexeieff (2, 3, 4, 5), Aragao (8), Behla (9), Beijerinck (11, 12), Brodsky (31), Bütschli (35), Chatton (42), Chatton and Lalung-Bonnaire (43), Dangeard (59), Frosch (93), Glaser (100), Hartmann (122, 123), Hartmann and Nägler (124), Nägler (228), Vahlkampe (311), Wasielewski and Hirschfeld (321), Wherry (322), Whitmore (323), and Zopf (337).

The morphology, physiology, etc., of protozoa have been studied by: Maupas (215), Kirchner and Blochman (164), Stein (292), Klebs (165), Krukenberg (192), Monton (221), Hartog and Dixon (125), Stole (295), Dallinger (57, 58), Kühn (191), Almquist (6), Schepilevsky (273), Stokvis (294), Holdhaus (138), Kleiber (166), Crampton (51), Straus (304) and Ogata (233).

An interesting phenomenon observed by Woodruff (333) and others is the so-called protozoan sequence, where certain dominant types are found in greatest abundance at one time, only to give way to another type. He studied the dominant types seeded in hay infusion, i. e., *Monad*, *Colpoda*, *Hypotrichida*, *Paramacium*, *Vorticella* and *Amabæ*. (*Colpoda cucullus* was present in the largest numbers.) A definite sequence does not occur at the middle or bottom of the infusion; the middle portion being tenanted chiefly by free-swimming forms brought by overcrowding from top and bottom. (All the forms except amœbæ are surface dwellers.) Cysts were found mostly at the bottom, immediately after a surface maximum had been reached. He concludes that food and specific excretion products are the most important determining factors in the observed sequence. Conjugation is resorted to in order to survive acute change of environment which precludes encystment.

In a later communication, Woodruff (334) maintains that *Paramecium* and *Hypotrichs* excrete substances toxic to themselves (specific), and these inhibit the rate of reproduction but do not affect other species of protozoa. Fine (80) states that the acidity of hay infusions is due essentially to bacteria, and that their efficiency in producing acid is governed by the concentration of the infusion in acid-yielding materials. The protozoa, he finds, play a relatively small part in the production of acid, but the protozoan sequence and the course of titratable acid possess an intimately mutual relation. Either may vary within wide limits, however, without appreciably affecting the course of the other.

Jacobs (147) has investigated the effect of CO_2 on protozoa. He finds that each of the twelve forms studied react to CO_2 in a characteristic way, and further, that each has a characteristic resistance, the most resistant form being *Colpidium colpoda*, which remains alive many hours, and the least resistant being *Coleps hirtus*, which is killed in a few minutes.

Some forms are killed outright, and very quickly at that. In others all movements are stopped in a few minutes, but death occurs relatively late, the powers of recovery being high (*Euglena*). All cases result in cessation of movement and death. In the same cell the contractile elements are quickly paralyzed (*Vorticella*) and vibratile structures (cilia, membranes, flagella) are much more resistant.

In ciliates, except *Vorticella*, recovery after complete cessation is impossible. In flagellates movement ceases long before the cell is permanently injured. The general effects of CO_2 on a cell are to cause (a) cessation of movements; (b) absorption of water and consequent swelling; (c) injury to cell wall, and (d) death and coagulation of the protoplasm.

Alexeieff (4) describes a flagellated stage in the evolution of *Amaba limax*. Specimens of flagellated *A. limax* were found in cultures of varying ages, during times of rapid multiplication. These forms were usually smaller than the non-flagellated forms. They were variable in shape, being ovoid, cylindrical, or pear-shaped. In the latter case the largest end was anterior and flagellated. The flagellæ are part of the nucleus, as in true flagellates, the nucleus being 3 to 4 μ in diameter. The cytoplasm is more dense than in the non-flagellated forms, and does not contain so many or such large vacuoles.

C. W. Wilson (325) has worked out the life-cycle of an amoeba (*Noegleria gruberi*), showing conclusively that it has a flagellate stage. Kofoid (177) employs this fact to point out that in determining the relative kinds and numbers of protozoa in soils it would be essential to know whether the flagellates so reported were true flagellates or only a stage in the life-cycle of amoebæ. Such a valuable suggestion will undoubtedly receive serious consideration in future soil protozoa studies.

Martin⁴ (211) reports on some protozoa from sick soils and gives an account of the life-cycle of a flagellated monad. He finds that the smaller amoebæ and flagellates play the most important rôle in the phenomena of sick soils, and that the most common limiting factor as regards the activity of protozoa in the soil is the average quantity of water. He argues that to be able to harm the bacteria, the protozoa must be satisfied with a very low percentage of water in the soil during their trophic life, or they must have the capacity of readily encysting, together with the capacity of reproducing with enormous rapidity as soon as the soil becomes saturated with the necessary amount of moisture and that they must be of small size. On sewage farms, any of the protozoa, even the larger ciliates, could harm the bacteria.

The author studied a roughly spherical animal, provided at its physiologically anterior end with a single, rather short, thick flagellum. Three types of culture plates were used—(a) rather dry; (b) fairly moist, and (c) quite moist. They did well in all at about 18° C., with the result that at the end of the third day the culture showed a large number of division figures. An encystation epidemic would set in—(a) at the end of a week; (b) at 12 days, and (c) when cultures were kept over 2 months without the appearance of encystation, this being due either to the osmotic effect upon the animal, diminution of food supply (bacterial flora), or the bacteria forming some toxin which is reacted against by encystation. Sub-cultures made from animals in a flourishing condition failed; whereas, from encystation they were successful. It seems that pronounced trophic activity proceeds conjugation, then encystment follows. A reserve body is formed in the posterior portion of the body. The cyst could be readily seen with iron-haematoxylin stain.

Martin and Lewin (214) believe that there are always some free living protozoa present in the active state in even relatively dry, poor soils, and that present methods are not adequate for their detection. They devised an air-blast method for that purpose, which is especially applicable to amoebæ. They further point out the necessity for rapid execution in methods depending upon the addition of water to the soil, because of the danger of excystation.

Some highly interesting facts can be gleaned from Doflein's "*Lehrbuch der Protozoenkunde*," already referred to in another connection. For example, he states that the motility of protozoa is affected by chemical substances in solution, salts, alkalis and acids, and that these cause a decrease in the surface of the pseudopodia, and increase the motility of the ciliates. Further, temperature, light, and mechanical agents all affect motility.

⁴The unenlightenment which makes war possible has deprived science of this able investigator. It is more than regrettable that his life and work should have been so abruptly discontinued.

He notes that distilled water kills protozoa, as does intensive sunlight. An instance of resistance of the cyst form is evidenced by the fact that *Colpoda* can come out of encystment after 14 to 16 months. He finds that most protozoa are killed at 38° to 42° C. but can withstand temperature below 0° C. if not frozen. *Euglena* in a free state is not injured by repeated freezings and most cysts endure freezing well. He corroborates the fact that *Colpoda*, etc., are bacteria-eaters and maintains that large protozoa cannot tolerate the presence of small ciliates and flagellates in the same culture medium.

Doflein refers to the work of Grosse-Allerman, who showed that *Amaba terricola* is killed after a few hours at 25° C.

Francé (85) states that from March to October the protozoa are active if other conditions are favorable. After that they encyst, as a result of frost or aridity. He also points out the significance of the "edaphons," as he terms them, in rendering the chemical and mechanical condition (by flocculation) more favorable.

Up to this point we have endeavored to bring together most of the important work which might throw light upon the problems of the soil protozoologist. The methods, as well as the activities of protozoa, have been discussed, and the all-important question regarding the function of the protozoa in the soil demands further attention.

Ehrenberg (69) was one of the first investigators to note the occurrence of infusoria in large numbers in rich soil. In 1836 Greef (111) described several species of *Amaba*, etc., from soil. In 1869 Rosenberg-Lipinsky (255) also referred to the occurrence of infusoria in soil and considered that they were probably of importance in regard to soil fertility. The first reference to the special function of soil protozoa is that of Breal (28) published in 1896. He found *Colpidium* active in the decomposition of plant constituents of the soil with the production of ammonia. The subsequent investigators have already been referred to in one connection or another. It will be remembered that Russell and Hutchinson maintain that protozoa destroy bacteria, yet it is remarkable to note that we have been unable to find, with but one exception to be considered at a later point, any investigation concerning itself with the actual ingestion of bacteria by protozoa.

Huntemüller (141) and Hoffman (137) have shown conclusively that water protozoa ingest typhoid bacteria. The former, experimenting with pure culture of typhoid bacteria and *Bodo ovatus* and *Bodo saltans*, found that these protozoa decreased the numbers of typhoid bacteria in water very markedly. They fed the protozoa with stained bacteria and obtained excellent micro-photographs showing the presence of the stained bacteria in the food vacuoles of the protozoa.

Calkins (36) has said that all protozoa ingest bacteria, with the exception of the parasitic forms, and those which live on other protozoa. Min-

chin (220), however, has recently stated that a number of protozoa are saprophytic in nature and obtain the food by absorption.

Gimingham (98) states that protozoa limit bacterial multiplication.

Thornton and Smith (308) have made a study of the successive but usually irregular developmental phases of certain fresh-water and soil protista. Soil flagellates as compared with *Euglena* are able to live in cultures to which organic compounds of varying natures have been added, this comparative impartiality being the result of the holozoic mode of nutrition and the development of the flagellates being dependent on the bacterial growth. It is said also that the presence of Miquel salts in the solution is necessary for the growth of the soil flagellates and for the proper development of the different types of bacteria upon which they feed.

Cunningham and Löhnis (54) in a valuable paper entitled "Studies on Soil Protozoa" have made an exceedingly careful and well-planned investigation of many aspects of soil protozoology. Their survey on bacterial solutions, etc., as media has already been described. It will be remembered that they also found a general sequence of development of the various groups of protozoa in the following order: flagellates, ciliates, and amœba. However, no association of certain protozoa with definite species of bacteria was noticed. Some of the protozoal species found were: Flagellates: *Oicomonas termo*, *Oicomonas dallingeri*, *Monas vivipara*, *Prowazekia terricola*, *Cyatromonas truncata*; Ciliates: *Colpidium colpoda*, *Colpoda cucullus*, *C. steinii*, *Oxytricha*, *Pellionella*, *Enchelys* sp., *Amœba* sp.

Cunningham and Löhnis quote Dallinger, who found that cysts of flagellates withstand 120° to 130° C., and Meunier (217), who found that *Colpoda* cysts were killed at 100° C. Tsujitani (310) found that 10 minutes at 60° C. killed all amœbæ, including cysts.

Cunningham and Löhnis also maintain that 60° C. represents the thermal death point of active protozoa, while 72° C. was found to kill all the cysts. The summary of their results on the study of the effect of heat upon active forms of protozoa and cysts is as follows:

	Death Points Active Forms	Cysts
Flagellates	44° C.	70-72° C.
Ciliates	54° C.	72° C.
Amœbæ	48° C.	72° C.

It might be added that they consider that 55° to 60° C. in the soil corresponds to 65° to 70° in culture solutions. In a later communication Cunningham (53) deals with the dilution method and its application to the enumeration of protozoa in soils, using the bacteriological method (previously described) and employing a medium of soil extract and 0.05

per cent K_2HPO_4 . To facilitate microscopic counting the soil extract is inoculated with a protozoa-free culture of bacteria (in bloodmeal solution), and incubated for 2 days before inoculation from the dilutions (which were 100, 300, 500, 750, 1000, 3000, 5000, etc.). It was noted that 5 days was as good an incubation period for protozoa as 30 days, though of course the results with such a method could not be considered absolute, but only approximate—one of the principal obstacles in the procedure being the adherence of the protozoa to particles of soil, and thus being carried over from dilution to dilution.

Adding 0.01 per cent of hydrochloric acid or caustic potash or chalk effected no improvement in the regularity of the results. Fifty-eight degrees C. was the temperature employed to distinguish between active and living forms, but it was soon found that many cysts, as well as living protozoa, are killed at this temperature.

As regards occurrence of protozoa in soils, flagellates predominate in number over amoebæ and ciliates. The effect of temperature is somewhat as follows: The protozoa are active in the soil at 22° C. but fall in total numbers and increase in cysts at 30° C. However, there are some active forms, such as *Balantiophorus*, at 30°. At 38° only a few amoebæ were noted. Below 8°, flagellates alone are present.

Cunningham records the effect of drying on reducing total numbers of protozoa. In cultures from saturated soils, flagellates alone were found, while at 70 per cent saturation and in dried soil amoebæ were found. Ciliates were seldom seen. Thus the author concludes that flagellates need a moist medium for their development, while amoebæ prefer a drier soil.

In investigating the influence of protozoa on the numbers of bacteria developing in ammonifying solution, saponinin was used to suppress protozoa, but was discarded. The method adopted was to inoculate 100-c.c. quantities of a 0.4 per cent bloodmeal solution (unfiltered) and 0.05 per cent K_2HPO_4 with a loopful of protozoa-free bloodmeal culture. After 2 days at 22° C. some of the flasks received in addition a loopful of bloodmeal culture containing protozoa from soil, so that from the beginning they contained more bacteria than the protozoa-free cultures. Bacterial counts were made after 10 and 20 days and it was found there was a quite marked reduction in the bacterial numbers as a result of the presence of protozoa in all six experiments, thus proving conclusively that in solution soil protozoa exercise a very decided limiting effect on the numbers of bacteria.

In determining the influence of protozoa on ammonification in solution tests, no definite conclusions could be established by the author.

Finally, protozoa were inoculated into soils partially sterilized with formalin. Two sets of experiments were mutually confirmatory, the latter of the two yielding the following results:

Inoculation	Millions per gram Bacteria	
1 c.c. Protozoa-free culture and 1 c.c. protozoa and 1 c.c. sterile H ₂ O.....	100	32
1 c.c. Protozoa-free culture and 1 c.c. protozoa and 1 c.c. sterile fleshmeal solution	133	77
1 c.c. Protozoa-free culture and 2 c.c. sterile H ₂ O	860
1 c.c. Protozoa-free culture and 1 c.c. sterile H ₂ O and 1 c.c. sterile fleshmeal solution	420	950
2 c.c. Sterile H ₂ O and 1 c.c. sterile fleshmeal solution.....
3 c.c. Sterile H ₂ O

The soil was sterilized with 5 c.c. formalin in 35 c.c. H₂O.

The conclusion is inevitable that the reduction in bacterial numbers in the soils inoculated with protozoa is very marked and lies well within the limits of experimental error, which strongly supports the contention of Russell and Hutchinson that soil protozoa are one of the limiting factors in soil fertility.

In a more recent investigation Kelley (160) has shown with cultivated and uncultivated soils that sterilization in the autoclave for 2 hours at 2 atmospheres affected these soils in such a way as to render them practically equal in regard to subsequent ammonification and brought about conditions toxic to nitrification in each instance; similar effects were produced by heating to still higher temperatures. In an interesting study he found that partial sterilization with heat at 98° C. and 4 per cent toluol and CS₂ greatly stimulated ammonification, which persisted usually for about 2 weeks only, followed then by a retardation in ammonification to a point below that which took place in untreated soil. Nitrification was prevented for a short time but later regained its activity, finally becoming more active than in the untreated soil. The reinoculation of partially sterilized soils with 5 per cent of the original soil in some instances caused a temporary reduction in the amount of nitrate and ammonia present, but this effect was not always permanent. Other effects of a different kind were also noted. He found, however, that not all protozoa were destroyed by the antiseptics employed. While Kelley does not consider his results to be in harmony with Russell's theory, though maintaining an open mind toward the problem, much of his data may be considered as supporting the latter. The necessity is indicated for considering partial sterilization phenomena as a resultant of a complexity of factors rather than such a simple explanation as protozoa.

The most recent contribution of special value both because of its scope and careful execution is that of Sherman (287, 288, 289). Using a soil extract medium, determinations made by using the dilution method indicated that the average fertile soil of Wisconsin (using 12 types) has a protozoan content approximating 10,000 per gram. Flagellates were the predominating type and *Colpoda cucullus* appeared to be the most widely distributed ciliate, occurring in numbers approximating 1000 per

gram. In order to determine whether soil protozoa are active, sterilized soil was inoculated with 0.1 per cent of normal soil known to contain 10,000 protozoa per gram and the following moisture contents: 11, 12, 15 and 22 per cent, maintained (17 per cent representing the optimum for this soil). After 15 days' incubation it was found that there was a multiplication of protozoa with the highest moisture content as well as with the subnormal content of 15 per cent. With the pure cultures these results were further corroborated. An important point in this connection, because of its bearing upon the validity of the entire investigation, is that when sterilized soil is inoculated with normal soil, the protozoan fauna rises in numbers above that of normal soil, just as does the bacterial flora. In other words, Sherman believes that it is probable that the microorganic balance remains about the same. It would appear that in addition to the legitimate objection which Sherman recognizes, and which is generally advanced by Russell, namely, that the medium is changed by drastic sterilization, another point might be advanced, to wit, that of species relationships. Thus certain groups of bacteria subject to destruction by protozoa in normal soil might be eliminated, while other groups of no importance, or even detrimental to protozoa, might be unduly multiplied. Thus the microorganic population would be numerically about the same but its physiological efficiency vastly different. Again, in employing "protozoa-free soil" which was made up of a mixed flora obtained from several different soils by the isolation of as many kinds of bacteria as could be obtained, a satisfactory qualitative flora would not involve an adequate quantitative flora, particularly with regard to species relationships. He is conscious of the limitation with regard to complexity of flora. In several tests soils free of protozoa contained greater numbers of bacteria than the corresponding soils which were inoculated with normal soil. The results were not considered as conclusive because of the differences in the two soils under consideration, inasmuch as it is definitely established that with an increase in complexity, the numbers of bacteria decreased. Experiments with soils containing protozoa and free from protozoa showed that the bacterial flora in the two soils behaved in exactly the same way when exposed to different temperatures or low moisture content. Thus the data obtained indicate that soil does not contain a biological factor which is harmful to bacteria. It may be stated, however, that no data are given concerning the actual number of protozoa present, while the number of bacteria is very high, namely, several hundred million per gram. If bacteria predominate, it is evident that protozoan activity will be seriously limited if not inhibited, as has been noted by other investigators. Furthermore, Sherman has employed only a low moisture content (8 per cent) in discussing the influence of that factor, hence this may not be considered as a very potent argument against the protozoan theory. Pure culture

tests with ciliates, on the other hand, showed that these organisms are very detrimental to bacteria in solutions. Since the ciliates are inactive in soil they are unable to affect the bacterial flora. Pure culture tests with types of active soil flagellates showed that these organisms were not capable of limiting the number of bacteria when acting in soil. One of the cultures, *Monas* sp., shown to be active in soil, has a strikingly harmful effect upon bacteria in soil extract. Treatment of soil with the ordinary amounts of volatile antiseptics (1 to 2 per cent) does not appear to simplify the protozoan fauna. A complex mixture of ciliates, flagellates and amoebæ is to be found in cultures made from soils partially sterilized with volatile antiseptics. As much as 10 per cent of CS_2 and toluene when added to soil fails to exterminate the protozoa entirely. It is regrettable that in this connection the author did not employ several soil types, thereby admitting of a broader generalization.

In partially sterilized soils the development of protozoa and bacteria subsequent to treatment with volatile antiseptics runs parallel. The re-inoculation of partially or completely sterilized soils by heat or antiseptics with 1 per cent of normal soil failed to decrease the number of bacteria. The treatment of soil with CS_2 at 37° C. gives a marked increase in the number of bacteria in the soils treated, which is not explained by the action of protozoa because of their presence in such small numbers in untreated soil at that temperature. Sherman's conclusion is that no evidence indicates that the beneficial effect of partial sterilization is due to the elimination of a biological factor which is harmful to bacteria.

Hills (132) in working out the relation of protozoa to certain groups of soil bacteria in Miami silt loam employed soil sterilized at 15 pounds pressure for 2 hours. Thus the conclusions arrived at are subject to the limitations found in Sherman's work. In a general way his results fall in line with those of the latter, namely, the presence of protozoa did not have any noticeable effect, detrimental or otherwise, on the processes of ammonification, nitrification and free nitrogen fixation in soil culture. In the case of the liquid cultures employed in the study of free nitrogen fixation, the conditions were at an optimum for the development of the protozoa and under those circumstances limited bacterial activity as evidenced by the harmful effect on the fixation of free nitrogen.

Greaves (109) found that the nitrogen-fixing powers of filtered soil extract (protozoa removed) are only slightly stimulated by arsenic, but as this occurs above the thermal death point of protozoa (as shown in another experiment), he concludes that the stimulation is mainly due to a removal of a thermolabile body which occurs in the soil, and not to any limiting effect of protozoa.

G. P. Koch (173, 174) found that the development of soil protozoa in artificial culture solutions varies with the kind of media employed, the quantity of soil used for inoculation, drying of the soil, different kinds

of soil and the temperature of incubation. Variations in the environment of protozoa were also studied in soil cultures (169). Employing the method of adding water to soil and examining directly he found that under ordinary greenhouse conditions small ciliates, flagellates and amœbæ are active in some soils, but their presence is very limited. Active protozoa do not seem to be present in field soils with a normal moisture content, and even when the moisture content is slightly supernormal.

Waksman (316, 317) found in a study of the environmental conditions governing the activities of protozoa that flagellates are the most common soil protozoa, and are found in greatest numbers at a depth of 4 inches. Below 12 inches the soil is practically free from protozoa. Soil protozoa did not appear to have any appreciable influence upon ammonification by bacteria, while bacterial numbers were decreased as a result of the development of protozoa under favorable conditions.

In a further investigation (317) of the effect of protozoa on bacteria it was found that the ammonifying efficiency was not paralleled by changes in bacterial numbers. Results on the application of antiseptics corroborated Sherman's conclusions. In most of his work the author has not dealt with the unchanged normal fauna; therefore, his results cannot be regarded as deprecatory to Russell's thesis.

C. M. Hutchinson (142) found that rapid nitrification takes place when green manure is placed in water and allowed to ferment, and that this is accompanied by the development of large numbers of ciliates, flagellates and amœbæ whose presence does not appear to be prejudicial to the activity of the ammonifying bacteria. He concedes, however, that this may be due to especially active multiplication of bacteria.

Cauda and Sangiorgi (38) have added to the knowledge of protozoa present in the soil by a study of the microfauna of soils of the rice-growing region. Fellers and Allison (79) have recently made a study of the kinds of protozoa found in the soils of New Jersey, and Peck (237) has also noted their presence in Hawaii.

Loew (203) mentions the wide distribution of *Colpoda cucullus* in acid stiff clay soils and sand dunes, as well as soils of Porto Rico, Japan, and the Alps.

THE PRESENT STATUS OF SOIL PROTOZOA AND SOIL STERILIZATION

It may be readily discerned from the review of the bulk of the literature dealing with soil protozoa and soil sterilization in the foregoing pages, that despite the work already done, comparatively little is known concerning the phenomena involved. Tracing the practice of soil sterilization by heat and disinfectants, it is to be observed that beyond recognizing the fact that crops are increased by such treatment, and that the chemical composition of the soil undergoes an alteration, together with a profound influence on the biological activities, data of a definite and

penetrating character are wanting. Briefly summing up the varied theories advanced to explain the phenomena of soil sterilization the following demand serious consideration.

1. In Koch's (171) theory of direct stimulation it is maintained that increased crop production is a result of the physiological effect of the sterilizing agency in stimulating plant growth directly. While several investigators have confirmed Koch's conclusions, nevertheless they are not widely accepted at the present day.

2. Hiltner and Störmer's (136) theory of "indirect" stimulation emphasizes the bacterial factor. These investigators maintain that there is a bacterial equilibrium in the soil which is altered by the introduction of sterilizing agencies. After the decimation of a vast number of bacteria has occurred, a marked development in numbers ensues, which is responsible for the additional available plant-food causing an increased crop yield. This theory has received the confirmation of many eminent investigators, and is still in vogue, although it hardly explains the phenomena observed, completely.

3. Liebscher's (197) view is that soil sterilization may be regarded in the same light as a nitrogenous fertilizer. This is not worked out in very great detail, but finds corroboration in most of the subsequent investigation.

4. Russell and Hutchinson's (265, 266) conclusions have been considered at some length and have been so frequently referred to, that it suffices to say at this point that they contend that sterilization eliminates a biological factor (protozoa) which is one of the limiting factors in soil fertility. This view, although having a profound influence upon all investigation in this field, has not been accepted by the majority of investigators working along the same lines.

5. Pickering (247, 248, 249) attaches the utmost significance to an alteration in the chemical composition of the soil—and proves that this change is largely responsible for increased plant growth.

6. Schreiner and his associates (277, 278, 279) also emphasize the chemical aspect of the problem, and contend that biochemical factors induce a change in the organic matter of the soil, releasing certain beneficial and harmful compounds which change the fertility of the soil.

7. Greig-Smith (113, 118) and others adhere to the bacterio-toxin hypothesis which considers that toxins and nutrients of the soil are alone concerned with the changes that occur when soils undergo sterilization.

In his latest paper (120) Greig-Smith contends that the traces of anti-septic remaining in the soil are responsible for increase in bacterial numbers and activities. It remains unquestionable that considerably more investigation will have to be carried out before any one of the above theories is accepted in an unqualified manner.

Considering the province of soil protozoology in its entirety, it immediately becomes apparent that this science is in its infancy—and is ur-

gently in need of suitable methods for making accurate investigation possible. As media, 1 per cent hay infusion, 3 per cent bloodmeal solution and soil extracts have proven most popular. In staining, picric acid (Kleinenberg) and iron haematoxylin (Delafield's) are most highly recommended. The "Blutkörperzählapparat" and the loop methods are employed for counting. A consideration of the inter-relation of protozoa and bacteria in normal soil remains practically a virgin field—although what little evidence there is obtainable points to the probability that the protozoa limit bacterial activity under conditions especially favorable to protozoan development.

Thus the scope of unsolved problems is considerably broader than the investigations already carried to completion. How to sterilize the soil without altering its chemical composition is an important though baffling problem. On the bacteriological side much can and needs to be done in determining the differences in the physiological efficiencies of the various groups of organisms in the soil and how they are affected by sterilization. Further, it is imperative to know what rôle the fungi play in soil fertility, and how they may be taken into consideration when it is desired to have bacteria or protozoa constitute the limiting factor. And in addition to an improvement and discovery of methods in soil protozoology it is essential to know more of the life-habits of these organisms, especially as regards the effect upon them of environmental conditions such as (a) the physical and chemical conditions in the soil, together with the effect of (b) air; (c) light; (d) heat; (e) moisture, reaction, gases, etc., as well as the mutual association with other biological factors. And finally, a matter demanding immediate study is the actual observation of pure cultures of protozoa acting singly and collectively upon pure cultures of bacteria, thus furnishing some definite basis for the investigation of soil protozoa as a factor in soil fertility.

With the solution of these problems will come a more profound understanding of the science of protozoology, a science which bids fair to take its place with soil bacteriology as furnishing a portion of the foundation essential for soil fertility investigations.

In conclusion, it is a pleasure to acknowledge the helpful suggestions of Dr. J. G. Lipman which have ever been at our disposal.

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⁵ Citations of literature in the original should be checked against this revision for unavoidable errata.

The writers wish to thank Mr. C. R. Woodward for his generous assistance in revising this bibliography, and the librarian of the U. S. Department of Agriculture, Washington, D. C., for furnishing biographical information.

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SOME EFFECTS OF SALT-TREATED SOILS ON ABSORPTION BY SEEDS¹

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In an experiment by Lipman and Gericke (6) on the effects of certain smelter wastes upon barley growth some interesting features of stimulation of growth were noted. This stimulation of growth resulted in an increase of total dry matter production for the plants grown in the treated over that of the plants grown on the check or untreated soils. In order to study more fully a certain phase of the results given in the paper above cited, the experiment herewith reported was carried out.

The purpose in mind in this experiment was to ascertain whether an increase due to absorption in the weight of good, viable seeds, planted in soils to which small amounts of certain toxic salts were added, would accrue over those seeds planted in untreated soils.

The method employed to secure the data, briefly described, is as follows:

A certain number of seeds which were carefully weighed were planted to a depth of 1 inch in tumblers containing 200 gm. of a light sandy soil. To this soil the respective salts were added in solution in quantities indicated in the tables, as parts per million of the dry weight of the soil. Uniform and optimum moisture and temperature conditions were maintained throughout the experiment. All tumblers were covered to prevent evaporation and to maintain a constant moisture condition. After intervals of 24 and 48 hours, as stated in the tables, the seeds were taken out of the soil, all adhering particles of soil were carefully removed with a camel's hair brush, and the seeds placed in weighing bottles, and weighed, as weight of seeds plus absorbed moisture. The weight of the seeds previously determined, subtracted from the weight of seeds obtained after removal from the soil gave the amount of moisture absorbed. The difference in percentage increase between differently treated seeds of the same series was ascribed to the influence of the salt, either as it affected the seed, the soil, or both. In no case was it found difficult to obtain determinations for the water absorbed, as long as no root development had occurred. Where root development had occurred, accurate determi-

¹ Received for publication October 17, 1916.

nations were precluded owing to the difficulty of separating adhering particles of soil from the roots. The determinations given in the tables are those of germinating seeds and not of plants with root development. The data given are for two determinations of each series, made at different times. The criterion of results is the difference in absorption of the seeds due to applications of varying amounts of salt in each series.

The data in Table I show that CuSO_4 added to the soil in applications ranging from 125 to 500 parts per million of the anhydrous salt produced an increase of absorption in the bean. Figures obtained for the barley do not show differences large enough to state conclusively that an increase in absorption of the seeds planted in the treated soil over those planted in the untreated soil has been obtained. The small differences, however, that have been obtained may indicate that the influence of CuSO_4 was in the direction to increase the absorption in barley. In case of the corn there was no increase of absorption of seeds due to the CuSO_4 treatment. The larger application of CuSO_4 retarded the increase of absorption of seeds in the three classes used.

The data in Table II show a distinct increase in absorption of seeds of the bean and barley planted for a 48-hour period in Na_2CO_3 -treated soil. The seed of the corn did not show any marked difference in absorption due to the Na_2CO_3 applications. However, what little difference was found, if at all indicative, shows that this salt has a tendency to increase absorption, interpreted by an increase of the weight of seeds planted in the treated soil over that planted in the untreated soil. The effect of Na_2CO_3 upon the seeds as indicated in the degree of absorption for the first 24-hour period was not nearly as large as that produced by the CuSO_4 within the same period of time, which may indicate the relative rate of reaction of the salts with the soils. Likewise, it may also indicate that the response of the seed is quicker to CuSO_4 than to Na_2CO_3 , when applied to the soils. However, as the absolute difference in increase of absorption produced by CuSO_4 and Na_2CO_3 is larger for the latter, after the 48-hour period, it is difficult to draw any conclusion as to the relative activity of chemical or physical reaction resulting from these two salts upon either the soil or the seeds planted therein.

Table III shows that small applications of NaCl to soils retarded the absorption of seeds planted in such soils. This salt affected all seeds in a similar way so far as relative weight due to absorption was concerned. The effects of the NaCl so far as the immediate and subsequent results shown in a 24 and 48-hour period are concerned, indicate that even small applications of this salt retarded water absorption.

Proceeding to a discussion of the results, in the tables we find that small amounts of salt added to soils affect viable seeds during the period of germination. The increase or decrease in the weight of the seeds planted in the salt-treated soils over that of the untreated soils may be

TABLE I
EFFECT OF CuSO_4 ON THE ABSORPTION OF WATER BY SEEDS IN SOIL

<i>Phaseolus nanus</i> , 1-day period				
No.	CuSO_4 added in parts per million	Weight of Seeds gm.	Weight of Seeds plus Absorption gm.	Per cent Absorption
1	0	1.3670	2.8330	207.24
2	125	1.3340	2.8600	214.63
3	250	1.3414	2.8000	209.04
4	500	1.3450	2.7816	206.65
5	750
1	0	1.3890	2.9906	215.30
2	125	1.4648	3.1652	225.37
3	250	1.4855	3.2845	221.10
4	500	1.4615	3.0846	211.05
5	750	1.4813	3.0771	207.73
<i>Phaseolus nanus</i> , 2-day period				
1	0	1.5072	3.9820	264.17
2	125	1.5042	4.2228	280.73
3	250	1.5004	4.1542	276.94
4	375	1.4986	4.0493	270.37
5	500	1.6390	4.0874	254.16
6	750
1	0	2.5414	6.7758	266.62
2	125
3	250	2.6298	7.8031	296.76
4	375	2.4330	7.3528	303.21
5	500	2.4094	7.3114	303.37
6	750	2.3728	6.1428	258.88
<i>Hordeum vulgare</i> , 2-day period				
1	0	1.0786	1.5890	147.32
2	125	1.0794	1.6028	148.49
3	250	1.0940	1.6214	148.20
4	500	1.1130	1.6474	148.01
5	750	1.1260	1.6443	145.95
1	0	0.9962	1.4816	149.72
2	125	0.9820	1.4744	150.14
3	250	1.0026	1.5500	152.60
4	500	1.0684	1.6056	150.28
5	750	1.1696	1.5798	145.16
<i>Zea Mays</i> , 2-day period				
1	0	3.6742	5.3564	146.33
2	125	3.8276	5.5152	144.09
3	250	3.7880	5.4586	144.10
4	500	3.6570	5.3212	145.51
5	750	3.7526	5.4044	144.00
1	0	3.6866	5.4188	147.00
2	125	4.0160	5.8844	146.50
3	250	3.8266	5.5300	144.51
4	500	3.6782	5.5084	149.76
5	750	3.7480	5.4442	145.26

TABLE II
EFFECT OF Na_2CO_3 ON THE ABSORPTION OF WATER BY SEEDS IN SOIL

<i>Phaseolus nanus</i> , 1-day period				
No.	Na_2CO_3 added in parts per million	Weight of Seeds gm.	Weight of Seeds plus Absorption gm.	Per cent Absorption
1	0	1.5408	3.0830	200.00
2	125	1.3652	2.7612	202.18
3	250	1.4200	2.8758	202.52
4	375	1.4106	2.8200	200.00
5	500	1.3454	2.7126	201.62
6	750	1.5432	3.0138	195.30
1	0	1.4835	3.1030	209.17
2	125	1.4802	3.0917	207.54
3	250	1.5587	3.2208	206.63
4	375	1.4469	3.1200	215.58
5	500	1.4917	3.1793	213.13
6	750	1.5600	3.0562	197.20
¹ <i>Phaseolus nanus</i> , 2-day period				
1	0	1.4374	4.1800	283.84
2	125	1.4044	4.3974	313.11
3	250	1.4646	4.6010	314.20
4	375	1.4564	4.7416	325.57
5	500	1.4284	4.7264	331.04
6	750	1.4374	4.5244	314.78
1	0	1.4442	3.1624	218.97
2	125	1.4460	3.5102	242.75
3	250	1.4682	3.5890	244.44
4	375	1.4526	3.5338	243.27
5	500	1.4770	3.4450	233.24
6	750	1.4700	3.5104	238.80
<i>Hordeum vulgare</i> , 2-day period				
1	0	1.1626	1.6780	144.32
2	125	1.0768	1.5708	145.83
3	250	1.9940	1.5828	148.54
4	500	1.0068	1.5256	151.52
5	750	1.0124	1.4882	146.08
6	1000	1.0176	1.3950	137.09
1	0	1.0240	1.5170	148.13
2	125	1.0088	1.5132	150.00
3	250	1.9940	1.4982	150.73
4	500	1.0892	1.6118	148.00
5	750	1.1400	1.6710	146.50
6	1000	1.0416	1.4650	140.65
<i>Zea Mays</i> , 2-day period				
1	0	4.4828	5.6712	126.50
2	125	4.4320	5.6552	127.60
3	250	4.1000	5.2200	127.02
4	500	4.3672	5.5216	126.21
5	750	4.1854	5.2556	123.93
1	0	4.4600	5.5962	125.46
2	125	4.2984	5.4240	126.02
3	250	4.2558	5.3626	126.71
4	500	4.6472	5.8536	125.98
5	750	4.4692	5.5704	124.64

¹Seeds used in this series were from two different lots, hence the difference in the amount of absorption.

TABLE III
EFFECT OF NaCl ON THE ABSORPTION OF WATER BY SEEDS IN SOIL

<i>Phaseolus nanus</i> , 1-day period				
No.	NaCl added in parts per million	Weight of Seeds gm.	Weight of Seeds plus Absorption gm.	Per cent Absorption
1	0	1.5900	3.1445	197.46
2	125	1.4990	2.9093	194.08
3	250	1.4766	2.9131	195.90
4	375	1.5277	2.9629	193.95
5	500	1.4130	2.7632	195.55
1	0	2.5798	5.2834	204.79
2	125	2.5456	5.1168	201.00
3	250	2.3756	4.7350	199.23
4	375	2.4374	4.8374	198.46
5	500	2.3854	4.6760	196.02
6	750	2.1324	3.8698	181.47
<i>Phaseolus nanus</i> , 2-day period				
1	0	1.4070	4.5090	320.40
2	125	1.4558	4.6300	318.03
3	250	1.4076	4.2066	298.85
4	375	1.4360	4.0566	282.49
5	500	1.3786	3.8340	278.10
6	750	1.6268	4.3540	267.89
1	0	1.4070	4.5000	319.83
2	125	1.4918	4.6750	313.38
3	250	1.4212	4.4923	316.09
4	375	1.4930	4.0658	272.32
5	500	1.5818	4.4045	278.44
6	750	1.3806	3.3067	246.76
<i>Hordeum vulgare</i> , 2-day period				
1	0	0.9770	1.6360	167.45
2	125	1.0000	1.6346	163.46
3	250	1.0722	1.7022	158.66
4	500	1.0873	1.6910	155.54
5	750	1.0232	1.6120	157.54
6	1000	1.0288	1.5992	155.44
7	2000	1.6544	1.6114	152.82
1	0	1.0530	1.8052	171.43
2	125	1.0280	1.7622	171.81
3	250	1.0616	1.6672	157.04
4	500	1.0932	1.7370	158.89
5	750	1.0286	1.6600	157.49
6	1000	1.0220	1.5750	154.20
7	2000	1.0574	1.5886	150.23
<i>Zea Mays</i> , 2-day period				
1	0	3.6494	5.3394	146.29
2	125	3.7064	5.3596	144.44
3	250	3.4662	5.0050	144.23
4	500	3.7208	5.3482	143.77
5	750	3.6622	5.2476	143.37

due to a difference in the quantity of water absorbed. That large amounts of soluble salts in a soil involving a high osmotic pressure of the soil solution can seriously interfere with the absorption of water by seeds or plants is well recognized. However, as the quantities of salts used in this experiment are so small as to render the consideration of osmotic pressure unimportant, the results obtained are very interesting.

Assuming that the increase of the weight of the seeds planted in the treated soil was due to an increase in the water absorption of the seeds, we can then imagine a force that may produce stimulation, that is, an actual increase of growth. Stimulation in case of germinating seeds would find its expression in increased water absorption which may increase the hydrolysis of the seed material, while in a growing plant stimulation would manifest itself rather in an increase of absorption and assimilation of plant-food resulting in a greater dry-matter production. The increase in absorption of water by the seeds through which stimulation of growth may be explained could result in two things: (a) an actual increase of growth per unit of time resulting in an actual increase of cell division in the individual, (b) an extension of the average magnitude and volume of the cells due to a greater water content which would necessarily result in the display of certain forces, such as increased turgidity, which may excite cell division and growth. The rôle that water plays in plant metabolism is undoubtedly subject to many variations which affect the chemical and mechanical reactions of the plant, and any variation in the absorption of water by the plant will affect the reactions of its life processes. Babcock (1) has shown that many of the chemical and bio-chemical reactions of plants can proceed only in a water solution, and that water, either as free water or in molecular combination, is necessary for the initiation of metabolic processes in seeds. The degree of viability of seeds, and the subsequent growth of the plants in many cases, have been found to be related, both to the absolute amount of available water and to the rate of its availability to the plant tissue.

Referring again to the experiment of Lipman and Gericke (6), together with their references, the conclusion seems reasonable that the increase in weight of seeds due to absorption which was influenced by some of the salts observed in this experiment, bears a direct relation to the stimulation of the plants noted in their experiment. The response of seeds to an application of salts to soils was very quick, and this must be considered significant as an index of the possibility of the immediate effects of fertilizer applications.

That weak solutions of salts, acids, and bases may increase the absorption of water by seeds in some cases has been shown by Dachnowski (2). The latter's results are in harmony with those of the writer in another experiment concerning absorption by seeds in solutions. How-

ever, seeds which were found to show an increase of weight, presumably due to an increase in the absorption of water, when planted in salt-treated soils, did not always follow the same tendency when placed in solutions of a given salt. Thus Table I shows that small applications of CuSO_4 to the soil increased the weight of the seeds planted therein over those planted in untreated soil, while the opposite was true of seeds placed in very weak CuSO_4 solution. That small applications of CuSO_4 may materially affect some of the plant-foods in the soil, and perhaps those of the soil solution in general, is shown by the work of Lipman and Burgess (4) in which they have been able to record a large increase in nitrate content of soils treated with small applications of CuSO_4 . The significance of an increase in nitrate content of a soil as an important factor to account for stimulation of plant growth cannot be overlooked, but whether an increase in nitrate content occurs in a 24-hour period (the one used in this case) is doubtful. This is so especially in view of the fact that Na_2CO_3 increases absorption, and Lipman (3) has found this salt decreases nitrification in soils.

Small applications of Na_2CO_3 to soils seem to be beneficial to plant growth according to the criterion of increased weight of seeds planted in such soils. This salt, important as one of the chief alkali salts of arid and semi-arid regions, has proven itself a stimulant to plants, if added in small quantities to normal soils, according to some of the former experiments of Lipman and Gericke (6). Seeds placed in aqueous solutions of Na_2CO_3 were found to increase in absorptive power.

Small applications of NaCl to soils tended to depress the increase of the weight of seeds of the three kinds tried, while the opposite was true in case of the NaCl solution. Dachnowski in case of seeds, Loeb (7) in case of muscle, and the writer, in unpublished results with seeds, all working with solutions, have found that weak NaCl solutions materially aid absorption. That the depression of the increase of weight of seeds in the present results was due to the action of NaCl upon the soil seems a reasonable conclusion. The inter-relationship between soil colloids and salts and the sphere of physico-chemical reactions of the soil, and their effect upon available water has been shown by Sharp (8) to be a factor of great importance.

The different effects upon the increase of weight of seeds, resulting from small applications of different salts, indicate a difference in chemical factors, both in soils and in plants. The relation of soil surface to soil water, the concentration of the soil solution, and the rate of movement of available water are known to affect the absorption and growth of plants. The effects of catalyzers, which hasten reactions, have been found to be important factors in plant life. The relation of these factors, due to the presence of salts applied to soils, constitutes an interesting phase in the greater problem of plant nutrition.

CONCLUSIONS

1. An experiment showing some of the effects upon seeds of small applications of CuSO_4 , Na_2CO_3 , and NaCl to the soils in which the seeds are planted is here reported.

2. Small applications of CuSO_4 to soil increased the absorption of the seeds of the bean and barley over that of the seeds planted in soil with no CuSO_4 . The effect of CuSO_4 upon the corn did not show differences large enough to indicate whether this salt increases or decreases absorption, when applied in small amounts. The larger application of CuSO_4 to soils decreased the absorption of all of the seeds used.

3. Small applications of Na_2CO_3 to soils increased the weight of all three kinds of seeds used in the experiment, through increased absorption.

4. Small applications of NaCl to soils decreased the absorption of water of all three kinds of seeds used.

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RELATION BETWEEN INDICATIONS OF SEVERAL LIME-REQUIREMENT METHODS AND THE SOIL'S CONTENT OF BASES¹

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INTRODUCTION

While almost any of the numerous lime-requirement methods which have been proposed are capable of showing distinct differences in indications as to "lime requirement" when tested with samples of the same soil before and after liming, it is very desirable to have a method the indications of which may be relied upon as being quantitative. The indications of practically all these methods may be given a quantitative expression—pounds per acre or parts per million of calcium carbonate or oxide required—but as two procedures rarely afford identical results for the same sample, none can be considered as giving correct indications.

Although it cannot be expected that the indications of any lime-requirement method will correspond to the optimum rate of application of lime to the field when considered from the practical standpoint, a study of these methods would seem to be justified from the fact that by the proper interpretation of the data obtained from a reliable lime-requirement method, an index to changes in a particular soil's supply of basic material may be obtained.

Differences in indications as to lime requirement afforded by a number of methods on samples of the same soil suggested the ideas that these methods measure different parts of the total base-absorbing capacity of the soil, or that they vary in efficiency in their indications of the total absorbent capacity. In order to secure some data on these points, laboratory experiments were conducted on two soils well supplied with basic material in forms other than carbonates. In addition, data obtained from analyses of soil from the 5-year rotation fertility experiment plots at the Ohio Experiment Station were considered in this connection.

¹ Received for publication October 11, 1916.

SOILS

Of the two soils employed for the laboratory experiment, Sample I is a Miami clay loam deficient in organic matter and is neutral to litmus paper; Sample II is a Clyde clay, black in color from the presence of a considerable accumulation of organic matter, and is strongly alkaline to litmus, although the calcium carbonate content is only about 0.02 per cent. Both of these samples represent the surface 6 inches of soil.

The Wooster silt loam, on which the fertility plots at this station are located, is naturally deficient in basic material and reddens blue litmus paper. The plots under discussion have been limed on their west halves only and data derived from differences in composition of samples taken to both the 6 and 12-inch depths are considered.

METHOD

Two-hundred-gm. portions of Samples I and II were extracted with 500 c.c. of hydrochloric acid of various definite concentrations ranging from centi-normal to normal. At the end of 5 hours' digestion at room temperature with frequent shaking, the mixtures of soil and solution were filtered, aliquots were taken, boiled and titrated with N/10 sodium hydroxide for residual acidity due to free acid, with methyl orange as an indicator, and for residual acidity due to iron and aluminum salts, with phenolphthalein as an indicator. The acid solutions were not further examined.

The soil on the filters was washed with distilled water until the washings gave no test for chlorides; the soil was then allowed to become air-dry, reground, and lime-requirement determinations made by the Hopkins method (2), the Hutchinson-MacLennan method (3), and the Vacuum method (1).

Bases Removed from Soils

The amounts of neutral salt-forming bases calculated to their equivalent in calcium carbonate and the amounts of acid salt-forming bases calculated to their equivalent in aluminum oxide extracted from 200-gm. portions of these soils by 500 c.c. of hydrochloric acid of various concentrations are shown graphically in figure 1, in which abscissae represent initial strength of acid and ordinates grams of base dissolved.

It may be seen from the graph that the solvent effects of the acid increase rapidly with increase in concentration up to a certain point, and thereafter more slowly; this effect is especially marked in the case of the Clyde clay. The extraction with 0.3 N. acid is seen to have removed the greater part of the readily attacked bases. Five-hundred c.c. of 0.3 N. HCl is equivalent to 7.5 gm. of CaCO_3 ; the equivalent of 3.3 gm. of CaCO_3 was removed by the acid at this concentration, leaving acidity in the solution equivalent to 4.2 gm. of CaCO_3 , which would make the end concentration of acid in this case not far from 0.2 N.

With the Miami clay loam, the initial concentration of acid necessary to extract the more easily soluble base is much lower—only about 0.05 N.—and the final concentration about 0.025 N. Beyond this point increases in the strength of acid used for the extraction cause comparatively slight

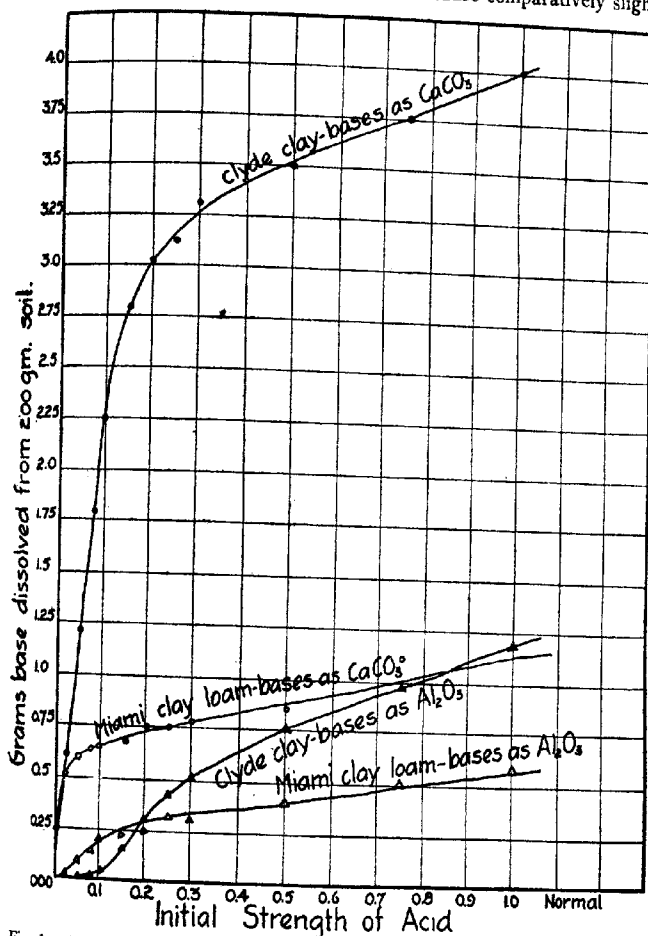


Fig. 1.—Amounts of bases dissolved from Clyde clay and Miami clay loam by various concentrations of hydrochloric acid.

increases in the amount of base extracted, so that the fifth normal acid-soluble bases may in this case also be taken as an approximation to the more easily soluble bases of the soil. These relationships are of interest, and indicate the value of fifth normal nitric-acid-soluble calcium and magnesium determinations in soil studies. As will be explained more fully later,

there is reason for the belief that the bases soluble in acid of this concentration approximate to the loosely held base which is so combined as to satisfy the lime requirement as determined by several of the methods studied, due consideration being given to the presence of carbonates.

Lime-Requirement Data of Acid-Extracted Soils

The data obtained from lime-requirement determinations on these acid-extracted soils are shown in graphic form in figures 2 and 3. In each case, abscissae represent calcium carbonate equivalent to the bases extracted from the soil, expressed as parts per 2,000,000 or, roughly, pounds per acre to a depth of 6 inches, and ordinates represent lime re-

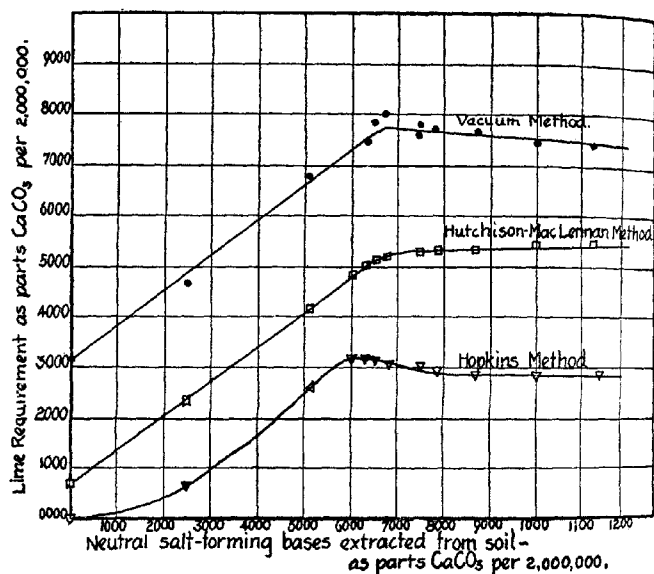


Fig. 2.—Diagram representing lime-requirement data of Miami clay loam determined by three methods.

quirements by the several methods, expressed as parts of calcium carbonate required per 2,000,000 parts of soil. It will be noticed that with both soils the Vacuum method has given the highest indications, as has been found to be the rule with unextracted soils; with the Miami clay loam, the Hutchinson-MacLennan method has apparently given superior indications of the actual amount of base removed by extraction, while with the Clyde clay, the Vacuum method has afforded better results in this respect. In fact, the latter method has exhibited very good indications with this soil up to a certain point, beyond which further removal of bases seems to affect the lime-requirement figures very slightly. The same effect is noticeable with all the methods; in the case of the Hopkins method there is a

slight decrease in the indications for lime requirement after the maximum is reached. Upon comparison of the graphs it will be noticed that the point at which the lime requirement becomes comparatively constant corresponds very closely to the point at which the extracted base tends to follow more closely increases in concentration of acid.

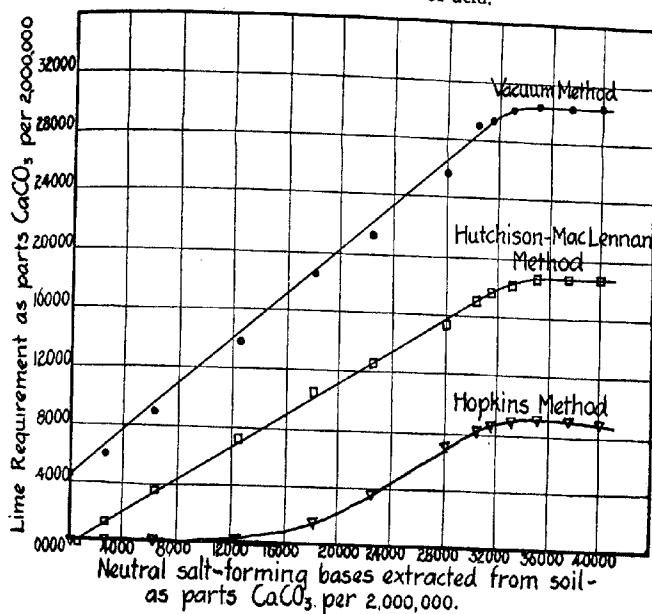


Fig. 3.—Diagram representing lime-requirement data of Clyde clay determined by three methods.

With both soils the Hutchinson-MacLennan method has given very consistent results, which are nearly parallel to although uniformly lower than those by the Vacuum method. As a method for comparative purposes the former is probably satisfactory. From theoretical considerations, the Vacuum method should afford the most accurate quantitative data. The fact that in the case of acid-extracted Miami clay loam the results obtained were on the whole less satisfactory than those by the Hutchinson-MacLennan method is difficult to explain.

The peculiar form of the curve drawn through the points representing data obtained by the Hopkins method indicate that this method is not well adapted to obtaining quantitative data, at least not with soils fairly well supplied with bases. The non-quantitative nature of this method is easily demonstrated in another way: considerable amounts of alkali may be added to the mixture of soil and potassium nitrate solution without reducing the indicated lime requirement proportionately to the amount of alkali added.

The possibility that the properties of the soil have been affected in an unexpected manner by acid extraction and subsequent washing and drying must be considered. The results obtained do not indicate that destruction of base-absorbent properties by the more dilute acid used for extraction has been appreciable. With the stronger acid, however, this effect has been distinctly noticeable. It is well shown by a sample of Wooster silt loam having a lime requirement of 275 pounds of calcium carbonate per acre by the Hopkins method, which was increased to 2965 pounds by extraction with fifth normal hydrochloric acid, but only to 1250 pounds by extraction with strong acid. On the other hand, but a very small part of the total increase in lime requirement exhibited by these acid-extracted soils is attributable to a direct absorption of acid or acid salts by the soil. This was shown by the relatively small amounts of chlorine found in the potassium nitrate extracts.

Extraction with a Solution of Carbon Dioxide

A sample of the same Miami clay loam used for the experiment described was extracted with a solution of carbon dioxide. Bases equivalent to 688 pounds calcium carbonate per acre were extracted as determined by titration of an aliquot of the extract with deci-normal acid and methyl orange indicator. In Table I lime-requirement data for this CO_2 -extracted sample are presented. The figures refer to pounds of calcium carbonate required per 2,000,000 pounds of soil.

TABLE I
LIME REQUIREMENT OF UNTREATED AND CO_2 -EXTRACTED SAMPLES OF THE
SAME SOIL

	Hopkins	Vacuum	Hutchinson- MacLennan
Untreated sample	13	3200	700
CO_2 -extracted sample	50	3400	1050

Relation between Fifth Normal Nitric-Acid-Soluble Bases and Indications of Several Methods for Lime Requirement

It has been mentioned that for the soils studied there would seem to be an approximately quantitative relationship between the fifth normal nitric-acid-soluble bases and that part of the soil's supply of base, which may be considered to be so combined as to satisfy the lime requirement as determined by several methods. In Table II, data tending to lend support to this opinion are presented. The soils studied include 5 plots of Section D, used in a 5-year rotation fertility experiment at this station, including composite samples from all the unfertilized plots on both the limed and the unlimed ends. Data for both 6 and 12-inch samples from these plots are available and in the case of the 6-inch samples results ob-

tained from the Veitch and the MacIntire (4) methods are included. These plots had been limed on one end only; the west halves received 1875 pounds of burned lime per acre in 1903, and 2000 pounds of ground limestone in 1909, making the total application equivalent to 5700 pounds of calcium carbonate. The samples were taken in 1912. The amounts of carbonate residual in the west halves of these plots are small, averaging about 210 pounds of calcium carbonate per acre for the plots considered here. With the exception of the plot receiving complete fertilizer with ammonium sulfate as carrier of nitrogen, the soil of which contains no carbonate, the west halves of all the plots considered are slightly alkaline by the Veitch test. For present purposes the average lime requirement by this method is considered to be zero for the west ends of the plots. The lime extension plots of the 5-year rotation received 6 and 13 tons of ground limestone equivalent to 11,805 and 25,575 pounds of calcium carbonate per acre in 1907. The samples were taken in 1912.

In the case of the 6-ton plot, an average of 2757 pounds of calcium carbonate per acre is residual in the first 12 inches of soil; for the 13-ton plots the corresponding figure is 9551. The data given for these heavily-limed plots are the averaged results from two samples, one taken on the manured and the other on the unmanured end of the plot. The data presented for the plots of Section D are the averaged results from 5 sets of samples, one set from each plot considered. The proper corrections for residual carbonates have been applied to the data for excess calcium soluble in fifth normal nitric acid in soil of the limed plots, and also to the data derived from determinations by the Hopkins method. The data in Table II are calculated to the equivalent in parts of calcium per two or four millions parts of soil (pounds per acre 6 or 12 inches) of the calcium, magnesium and lime requirements as determined, in order to simplify comparison.

TABLE II
LIME REQUIREMENT OF FOUR SOILS EXPRESSED IN POUNDS OF CALCIUM PER ACRE

Series of Plots	Excess lime requirement by several methods, unlimed over limed ends of plots					Excess bases soluble in fifth normal nitric acid, limed over unlimed ends of plots
	Hopkins	Veitch	MacIntire	Hutchinson- MacLennan	Vacuum	
Sec. D, 5-yr. rotation, 0-6 inches..	622	913	521	543	1081	952
Sec. D, 5-yr. rotation, 0-12 inches.	1054	1969	1524
Lime Extension, 6 tons, 0-12 inches	1500	1919
Lime Extension, 13 tons, 0-12 inches	1964	1995

As may be seen by reference to Table II, the Hopkins method has given no better results with these samples of field-treated soils than would be expected from its indications as to loss of bases from the acid-extracted soils.

The Veitch method appears to have afforded excellent results, although the number of experiments is too small for drawing any very definite conclusions. Results by the MacIntire and Hutchinson-MacLennan methods, both of which employ a solution of calcium bi-carbonate as the carrier of base, appear to be only half the proper value. The Vacuum method has been studied at greatest length; the results obtained, while not remarkably close, indicate that this is the most reliable method of those studied, which is at the same time universally applicable.

It has been shown that for the Clyde clay and the Miami clay loam used in the extraction experiments, and for the Wooster silt loam in field experiments, there is an approximation to a quantitative relationship between differences in lime requirement as determined by the Vacuum method and differences in content of neutral salt-forming bases soluble in fifth normal acid as usually determined, the presence of carbonates being considered.

If the fifth normal nitric-acid-soluble bases not accounted for by the presence of carbonates are regarded as corresponding to base-absorbing capacity already satisfied, and the indicated lime requirement to residual base-absorbing capacity, an index of the extent to which the soil's total base-absorbing capacity is satisfied may be obtained.

In Table III data for a number of soils used in outdoor pot experiments are presented, together with the effect upon crop yields of an application, according to the indications of the Vacuum method, of precipitated calcium carbonate. Each soil and its subsoil are represented by 10 pots 20 inches in diameter sunk in the ground; 5 pairs of pots are variously fertilized, with and without lime. The reaction to litmus, the content and requirement by the Vacuum method of calcium carbonate, and the composition with respect to fifth normal nitric-acid-soluble calcium and magnesium of the original soil are given. In the column headed "Per cent total lime requirement satisfied" the data for soluble bases, carbonates and lime requirement are treated as outlined in a preceding paragraph, that is, after calculating all quantities involved into the corresponding percentages of calcium, the lime requirement as indicated by the Vacuum method is divided by the sum of the same and the fifth normal nitric-acid-soluble calcium and magnesium minus base corresponding to carbonate present, and the quotient expressed as per cent of total lime requirement satisfied. In the column headed "Effect of lime," average crop yields of the five limed and the five unlimed pots of each soil are compared: "+" indicates an increase for lime, and "-" a decreased or sta-

tionary yield. The pots were limed and fertilized but once, and all crops were grown to maturity.

The data presented in Table III require little comment. Attention is directed to the fact that the two neutral soils have more than 75 per cent of their total base-absorbing capacity satisfied, and that the three alkaline soils contain but a trace of carbonates and are approximately 90 per cent saturated with bases. An application of lime has been beneficial to the

TABLE III
LIME-REQUIREMENT DATA OF SOILS USED IN OUTDOOR POT EXPERIMENTS

	Per cent N/5 HNO ₃ soluble		Per cent CaCO ₃ Present	Per cent CaCO ₃ Required (Vacuum)	Per cent Total Lime- Requirement Satisfied	Litmus Test	Effect of Lime		
	Ca.	Mg.					Soybeans 1913	Wheat 1914	Clover 1915
Clermont silt loam, Clermont Co.	0.085	0.012	0	0.242	52	Acid	+	+	+
Clyde clay, Paulding Co.525	.045	.023	.214	87	Alk.	—	+	+
Upshur clay, Washington Co.221	.034	0	.360	66	Acid	—	+	+
Dunkirk sandy loam, Erie Co.047	.008	.007	.149	50	Acid	+	—	+
Clyde sandy loam, Lucas Co.155	.021	0	.158	75	Acid	+	+	+
Volusia silt loam, Ashtabula Co.061	.008	0	.618	23	Acid	+	+	+
Dunkirk gravelly loam, Ashtabula Co.079	.010	0	.353	40	Acid	+	+	+
Clyde clay loam, Lucas Co.455	.041	.003	.144	90	Alk.	+	+	—
Wooster silt loam, Wayne Co.076	.016	0	.237	52	Acid	+	+	+
Dunkirk sand, Lucas Co.023	.007	0	.015	85	Neutral	—	+	+
Miami clay loam, Montgomery Co. ..	.134	.025	0	.139	76	Neutral	+
Trumbull clay loam, Cuyahoga Co.085	.024	0	.600	34	Acid	+
Miami clay loam, Miami Co.107	.019	.005	.253	58	Acid
Clyde clay loam, Miami Co.543	.033	.032	.179	89	Alk.

clover crop in every case save one; the average yield of clover on the 5 limed pots of the Clyde clay loam was slightly below that of the 5 unlimed pots. This is the most basic soil included in the series. The acid soils are all less than 76 per cent saturated with calcium; some of the figures are very low, but a considerable range is exhibited. Further studies may demonstrate that the relationships described do not hold for the majority of soils, but these observations are offered as being suggestive.

SUMMARY

Many of the lime-requirement methods hitherto proposed have proved to be of limited usefulness for the reason that their indications are not quantitative.

Experiments upon acid-extracted and washed soils and upon field-treated soils demonstrate that the Vacuum method is, of the number studied, the most nearly quantitative method which is at the same time universally applicable.

The idea of determining the per cent of total lime requirement satisfied is offered as a means of comparing the condition of basicity of different soils, and as an aid in the solution of other soil problems.

The limited amount of experimental data available indicates that there may be some relation between the per cent of total lime requirement satisfied and the reaction of the soil to litmus, carbonates being practically absent.

Crop yields in pot experiments have not indicated that an application of precipitated calcium carbonate according to the indications of the Vacuum method, which is considered to represent saturation with calcium, is harmful in other than exceptional cases.

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'THE EFFECT OF SOME ACIDS AND ALKALIS ON SOIL BACTERIA IN THE THE SOIL SOLUTION'

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INTRODUCTION

The determination of the effect of acids and alkalis on the micro-flora of the soil is of immense practical and theoretical value for the explanation of the complex problems of soil fertility. Little or no attention has been directed to the study of changes in the bacterial flora of the soil solution at different acid and alkaline conditions. Such studies would aid materially to ascertain the resisting power of soil bacteria to the unfavorable conditions of reaction and would lead to the knowledge of the limits of reaction between which soil bacteria are most active.

The complexity of the soil mass, with its manifold and constantly changing processes, causes many difficulties in analytical interpretation of the results due to the chemical interaction occurring between the added compounds and the solid and liquid phases of the soil. To eliminate the effect due to the solid phase of the soil the liquid phase of the soil was extracted from it by the paraffin oil method (18) and used alone for the study of the effect of reaction on the soil bacteria.

Kossowich (14), Gedroitz (7), Harris (9), Abbot, Conner and Malley (1), Brown, Howe and Sar (2), and Schreiner and Skinner (19), have shown that toxicity of soil might be due to several factors, as free organic and inorganic acids and acid salts, adsorption of bases and other substances of organic or inorganic nature, or excessive quantities of alkali salts in the soil.

Harrison and Barlow (10), Klaeser (13), Fabricus and Feilitzen (6), Lipman, J. G., and Fraps (16), Lipman, C. B. (15), Hall, Miller and Gimingham (8), and Temple (20), present facts to show that the important groups of soil bacteria develop best on slightly alkaline media, with exception of *Pseudomonas radicola* associated with acid-tolerating crops (4).

¹ An abstract of the thesis submitted for the degree of Master of Science.

Received for publication December 13, 1916.

EXPERIMENTAL

The soil solutions used in our studies were extracted from rich sandy loam and sandy soils. The solutions were adjusted to various degrees of reaction with N/100 NaOH and HCl, methyl red being used as an indicator for titration, and 10-c.c. quantities were placed in 100 gm. of pure sterile quartz sand. The moisture content of the cultures was adjusted about every 10 days. The counts of bacteria in the soil solution were made upon sodium asparaginate agar (5) after 7 days of incubation at a temperature varying between 19° and 23° C.

The Effect of Hydrochloric Acid on Soil Bacteria

The soil solution was extracted from sandy soil on which alfalfa had been growing for several years. The extracted solution was adjusted to neutral, N/1200 acid, and N/446.4 alkaline (original reaction of the soil solution) conditions. The changes occurring in the number of bacteria under different reactions of the cultures are well expressed by the curves in figure 1. In cultures with N/446.4 alkaline reaction the development of bacteria was slight up to the end of the eighteenth day of incubation.

The greatest increase of bacteria in these cultures occurred at about the thirty-sixth day of incubation. By the sixtieth day of incubation the reaction had become acid and in parallel with the change of the reaction the number of bacteria had decreased. The cultures with neutral reaction supported development of bacteria feebly for a short period. In the cultures with the N/1200 acid reaction, decrease in the number of bacteria was sudden. After the eighteenth day of incubation the number of bacteria in these cultures was reduced by 98.9 per cent when the number of bacteria in N/446.4 alkaline reaction cultures was taken for comparison.

With the increase in the age of the cultures the reaction underwent gradual change. It took about 56 days to neutralize the alkaline reaction and produce enough acid to destroy about half of the bacteria. The cultures with neutral reaction indicated slight acidity at the eighteenth day of incubation. The destruction of bacteria in these cultures was gradual. In the N/1200 acid cultures the decrease in the number of bacteria was abrupt and sudden.

As a resumé of this experiment interesting suggestions can be made. First, under the conditions of our experiment, the cultures with alkaline and neutral reaction underwent gradual change from an alkaline to a neutral and from a neutral to an acid condition. Second, with the change in the reaction there occurred a gradual increase in the number of bacteria up to the point where the reaction was barely alkaline. Third, when the reaction became neutral and later acid, the number of bacteria decreased. Fourth, at no time could 98.9 per cent of the soil bacteria from a sandy soil endure an acid reaction of N/1200 hydrochloric acid.

The Effect of Sulfuric Acid on Soil Bacteria in the Soil Solution

The effect of various concentrations of sulfuric acid on soil bacteria, as summarized by the curves in figure 2, was in general the same as that of hydrochloric acid.

The soil solution was extracted from rich sandy loam soil. The original reaction of the soil solution titrated N/1040 alkaline to methyl red. The solution was adjusted to N/990 and N/412 alkaline, neutral, and N/1200, N/1400, N/2164 and N/2840 acid conditions. The cultures with

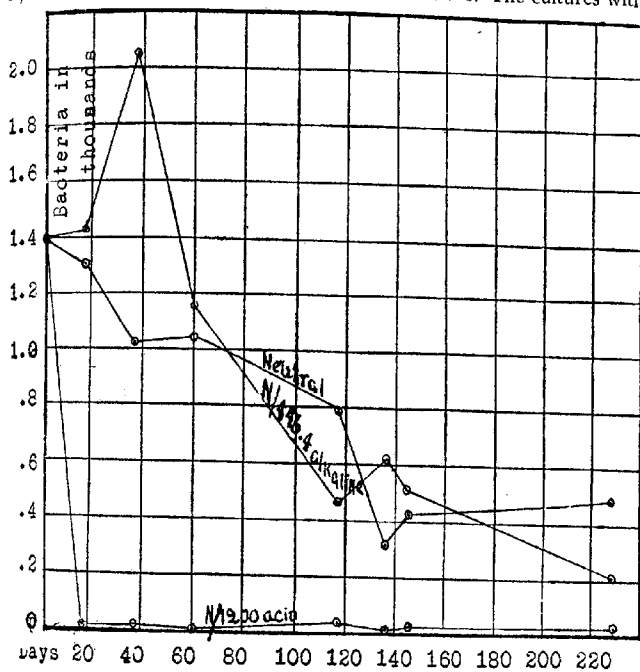


Fig. 1.—The effect of reaction on soil bacteria from a sandy soil.

alkaline reaction permitted the most intensive growth of bacteria until the medium became acid. However, the rate of growth was not the same in cultures with different OH-ion concentration. The increase in the number of bacteria in cultures with N/1040 OH-ion concentration was most rapid and reached the climax of growth after the tenth day of incubation. The development of bacteria in the cultures with N/990 OH-ion concentration reached the climax of the growth after the sixteenth day of incubation. Thus the slightly higher OH-ion concentration retarded the growth for 6 days. If in this connection the curve of N/412 OH-ion concentration is

studied, it is seen that the increase in the number of bacteria in these cultures in 6 days was only about half as rapid as in the cultures with N/1040 alkaline reaction. However, after the sixth day of incubation the increase was more rapid and continued to increase above the number of bacteria in N/990 alkaline reaction cultures.

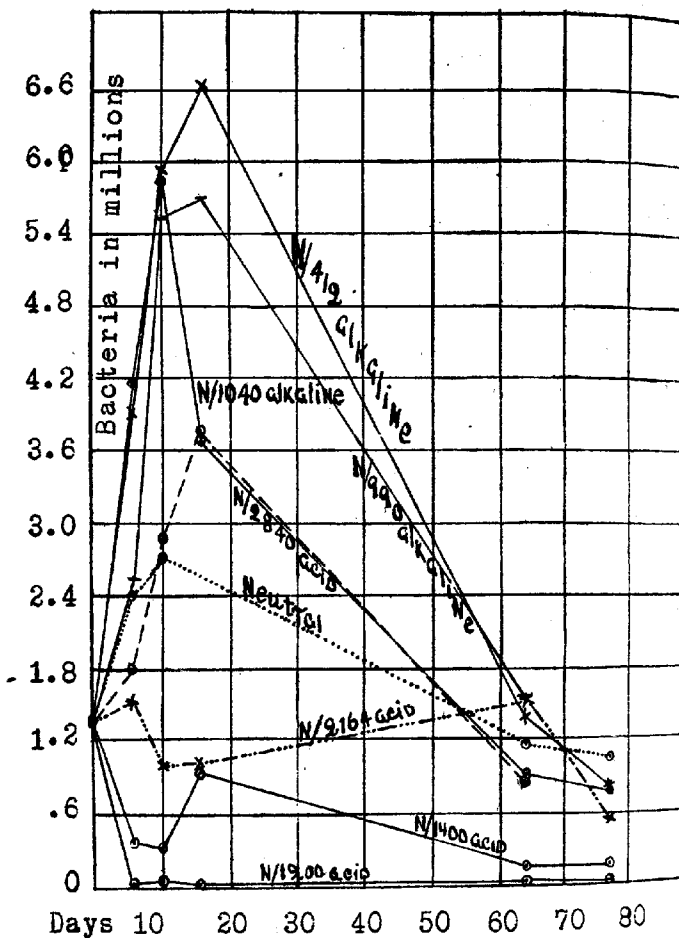


Fig. 2.—The effect of reaction on soil bacteria from a sandy loam soil.

To account for the unequal rate of the growth of soil bacteria in the different alkaline cultures we must suppose that there is a definite relation between the reaction of the soil solution and the development of soil

bacteria. The growth of bacteria at the beginning was slightly inhibited in the cultures with N/990 alkaline reaction. After 6 days of incubation this reaction underwent slight neutralization and, consequently, the number of bacteria was on the increase at the end of the sixteenth day of incubation, while in the cultures with N/1040 alkaline reaction the solution had passed the neutral point at the tenth day and the number of bacteria was decreasing. The growth of bacteria in the N/412 alkaline reaction cultures was strongly inhibited at the end of the sixth day. With the increase in the incubation period the reaction became weaker. In parallel with the decrease of OH-ion concentration the number of bacteria increased and reached the climax of development when the reaction was in the neighborhood of N/1000 alkaline.

The cultures adjusted to the neutral condition permitted only slight development of bacteria. When the reaction in these cultures became acid the number of bacteria dropped down rapidly.

The effect of the acid reaction on soil bacteria from a rich sandy loam soil was manifested according to the H-ion concentration in the cultures. The H-ion concentration of N/1200 sulfuric acid destroyed about 99.6 per cent of bacteria. In N/1400 H-ion concentration cultures about 7 per cent of bacteria survived. N/2164 H-ion concentration inhibited the growth to the extent to maintain the same number of bacteria for about 67 days. An H-ion concentration less than N/2164 permitted a slight development of bacteria. In the lowest H-ion concentration, N/2840, the number of bacteria was less by about 43 per cent than in cultures with N/412 OH-ion concentration after 16 days of incubation.

The sensitiveness of soil bacteria to an acid condition of the medium could be well emphasized by comparing the sensitiveness of plants towards acidity of the medium. Loew (17), Heald (12), Cameron and Breazeale (3), Hartwell and Pember (11) and Gedroitz (7) give the following H-ion concentrations for "toxic limits" to Indian corn and barley seedlings:

	Indian Corn Seedlings			Barley Seedlings	
	Loew	Heald	Cameron Breazeale	Hartwell Pember	Gedroitz
Hydrochloric Acid ..	N/512	N/3200	N/3000	N/5000	N/1250
Sulfuric Acid	N/512	N/3200	N/3000	N/5000	N/1250

The lowest H-ion concentration (N/2840) inhibited the growth of 42 per cent of the bacteria, which is close to Cameron and Breazeale's "toxic limit" for Indian corn seedlings. From the foregoing data it appears that soil bacteria under conditions of our experiment, were highly subject to the harmful effect of acid even in concentration at which Indian corn seedlings were only slightly affected.

SUMMARY

1. The development of the general flora of the soil bacteria from sand and sandy loam soils, when studied in the soil solution of sand cultures, was retarded if the reaction of the medium had a higher OH-ion concentration than N/1000.
2. The development of soil bacteria was inhibited when the reaction of the medium became neutral.
3. The H-ion concentration of N/1200 hydrochloric and sulfuric acids was germicidal to about 99.2 per cent of soil bacteria under the conditions of our experiment.
4. With H-ion concentration of N/2164 the rate of multiplication of soil bacteria from sandy loam corresponded to the rate of destruction so that there was no change in the total number of bacteria.
5. The H-ion concentration of N/2840 inhibited the growth of 43 per cent of soil bacteria when compared with the growth of soil bacteria in the medium with N/412 OH-ion concentration.
6. The reaction of alkaline cultures was gradually neutralized and later became acid.
7. When the "toxic limit" of acids towards Indian corn seedlings was compared with the toxic limit of about the same concentration of the same acids on the general flora of the soil bacteria from sand and sandy loam soils, the soil bacteria were injured to the extent of 43 per cent.

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